APPENDICES

APPENDICES (CONTINUED)

APPENDIX A

INDEPENDENCE, RANDOMIZATION, AND OUTLIERS

1. STATISTICAL INDEPENDENCE

1.1 Dunnett's Procedure and the t test with Bonferroni's adjustment are parametric procedures based on the assumptions that (1) the observations within treatments are independent and normally distributed, and (2) that the variance of the observations is homogeneous across all toxicant concentrations and the control. Of the three possible departures from the assumptions, non-normality, heterogeneity of variance, and lack of independence, those caused by lack of independence are the most difficult to resolve (see Scheffe, 1959). For toxicity data, statistical independence means that given knowledge of the true mean for a given concentration or control, knowledge of the error in any one actual observation would provide no information about the error in any other observation. Lack of independence is difficult to assess and difficult to test for statistically. It may also have serious effects on the true alpha or beta level. Therefore, it is of utmost importance to be aware of the need for statistical independence between observations and to be constantly vigilant in avoiding any patterned experimental procedure that might compromise independence. One of the best ways to help ensure independence is to follow proper randomization procedures throughout the test.

2. RANDOMIZATION

2.1 Randomization of the distribution of test organisms among test chambers and the arrangement of treatments and replicate chambers is an important part of conducting a valid test. The purpose of randomization is to avoid situations where test organisms are placed serially into test chambers, or where all replicates for a test concentration are located adjacent to one another, which could introduce bias into the test results.

2.2 An example of randomization of the distribution of test organisms among test chambers, and an example of randomization of arrangement of treatments and replicate chambers are described using the Fathead Minnow Larval Survival and Growth test. For the purpose of the example, the test design is as follows: five effluent concentrations are tested in addition to the control. The effluent concentrations are as follows: 6.25%, 12.5%, 25.0%, 50.0%, and 100.0%. There are four replicate chambers per treatment. Each replicate chamber contains ten fish.

2.3 RANDOMIZATION OF FISH TO REPLICATE CHAMBERS EXAMPLE

2.3.1 Consider first the random assignment of the fish to the replicate chambers. The first step is to label each of the replicate chambers with the control or effluent concentration and the replicate number. The next step is to assign each replicate chamber four double-digit numbers. An example of this assignment is provided in Table A.1. Note that the double digits 00 and 97 through 99 were not used.

TABLE A.1. RANDOM ASSIGNMENT OF FISH TO REPLICATE CHAMBERS EXAMPLE ASSIGNED NUMBERS FOR EACH REPLICATE CHAMBER

Assigned Numbers	Replicate Chamber
01, 25, 49, 73	Control, replicate chamber 1
02, 26, 50, 74	Control, replicate chamber 2
03, 27, 51, 75	Control, replicate chamber 3
04, 28, 52, 76	Control, replicate chamber 4
05, 29, 53, 77	6.25% effluent, replicate chamber 1
06, 30, 54, 78	6.25% effluent, replicate chamber 2
07, 31, 55, 79	6.25% effluent, replicate chamber 3
08, 32, 56, 80	6.25% effluent, replicate chamber 4
09, 33, 57, 81	12.5% effluent, replicate chamber 1
10, 34, 58, 82	12.5% effluent, replicate chamber 2
11, 35, 59, 83	12.5% effluent, replicate chamber 3
12, 36, 60, 84	12.5% effluent, replicate chamber 4
13, 37, 61, 85	25.0% effluent, replicate chamber 1
14, 38, 62, 86	25.0% effluent, replicate chamber 2
15, 39, 63, 87	25.0% effluent, replicate chamber 3
16, 40, 64, 88	25.0% effluent, replicate chamber 4
17, 41, 65, 89	50.0% effluent, replicate chamber 1
18, 42, 66, 90	50.0% effluent, replicate chamber 2
19, 43, 67, 91	50.0% effluent, replicate chamber 3
20, 44, 68, 92	50.0% effluent, replicate chamber 4
21, 45, 69, 93	100.0% effluent, replicate chamber 1
22, 46, 70, 94	100.0% effluent, replicate chamber 2
23, 47, 71, 95	100.0% effluent, replicate chamber 3
24, 48, 72, 96	100.0% effluent, replicate chamber 4

2.3.2 The random numbers used to carry out the random assignment of fish to replicate chambers are provided in Table A.2. The third step is to choose a starting position in Table A.2, and read the first double digit number. The first number read identifies the replicate chamber for the first fish taken from the tank. For the example, the first entry in row 2 was chosen as the starting position. The first number in this row is 37. According to Table A.1, this number corresponds to replicate chamber 1 of the 25.0% effluent concentration. Thus, the first fish taken from the tank is to be placed in replicate chamber 1 of the 25.0% effluent concentration.

2.3.3 The next step is to read the double digit number to the right of the first one. The second number identifies the replicate chamber for the second fish taken from the tank. Continuing the example, the second number read in row 2 of Table A.2 is 54. According to Table A.1, this number corresponds to replicate chamber 2 of the 6.25% effluent concentration. Thus, the second fish taken from the tank is to be placed in replicate chamber 2 of the 6.25% effluent concentration.

TABLE A.2. TABLE OF RANDOM NUMBERS (Dixon and Massey, 1983)

10 09 73 25 33	76 52 01 35 86	34 67 35 43 76	80 95 90 91 17	39 29 27 49 45
37 54 20 48 05	64 89 47 42 96	24 80 52 40 37	20 63 61 04 02	00 82 29 16 65
08 42 26 89 53	19 64 50 93 03	23 20 90 25 60	15 95 33 47 64	35 08 03 36 06
99 01 90 25 29	09 37 67 07 15	38 31 13 11 65	88 67 67 43 97	04 43 62 76 59
12 80 79 99 70	80 15 73 61 47	64 03 23 66 53	98 95 11 68 77	12 27 17 68 33
66 06 57 47 17	34 07 27 68 50	36 69 73 61 70	65 81 33 98 85	11 19 92 91 70
31 06 01 08 05	45 57 18 24 06	35 30 34 26 14	86 79 90 74 39	23 40 30 97 32
85 26 97 76 02	02 05 16 56 92	68 66 57 48 18	73 05 38 52 47	18 62 38 85 79
63 57 33 21 35	05 32 54 70 48	90 55 35 75 48	28 46 82 87 09	83 49 12 56 24
73 79 64 57 53	03 52 96 47 78	35 80 83 42 82	60 93 52 03 44	35 27 38 84 35
98 52 01 77 67	14 90 56 86 07	22 10 94 05 58	60 97 09 34 33	50 50 07 39 98
11 80 50 54 31	39 80 82 77 32	50 72 56 82 48	29 40 52 42 01	52 77 56 78 51
83 45 29 96 34	06 28 89 80 83	13 74 67 00 78	18 47 54 06 10	68 71 17 78 17
88 68 54 02 00	86 50 75 84 01	36 76 66 79 51	90 36 47 64 93	29 60 91 10 62
99 59 46 73 48	87 51 76 49 69	91 82 60 89 28	93 78 56 13 68	23 47 83 41 13
65 48 11 76 74	17 46 85 09 50	58 04 77 69 74	73 03 95 71 86	40 21 81 65 44
80 12 43 56 35	17 72 70 80 15	45 31 82 23 74	21 11 57 82 53	14 38 55 37 63
74 35 09 98 17	77 40 27 72 14	43 23 60 02 10	45 52 16 42 37	96 28 60 26 55
69 91 62 68 03	66 25 22 91 48	36 93 68 72 03	76 62 11 39 90	94 40 05 64 18
09 89 32 05 05	14 22 56 85 14	46 42 75 67 88	96 29 77 88 22	54 38 21 45 98
91 49 91 45 23	68 47 92 76 86	46 16 28 35 54	94 75 08 99 23	37 08 92 00 48
80 33 69 45 98	26 94 03 68 58	70 29 73 41 35	53 14 03 33 40	42 05 08 23 41
44 10 48 19 49	85 15 74 79 54	32 97 92 65 75	57 60 04 08 81	22 22 20 64 13
12 55 07 37 42	11 10 00 20 40	12 86 07 46 97	96 64 48 94 39	28 70 72 58 15
63 60 64 93 29	16 50 53 44 84	40 21 95 25 63	43 65 17 70 82	07 20 73 17 90
61 19 69 04 46	26 45 74 77 74	51 92 43 37 29	65 39 45 95 93	42 58 26 05 27
15 47 44 52 66	95 27 07 99 53	59 36 78 38 48	82 39 61 01 18	33 21 15 94 66
94 55 72 85 73	67 89 75 43 87	54 62 24 44 31	91 19 04 25 92	92 92 74 59 73
42 48 11 62 13	97 34 40 87 21	16 86 84 87 67	03 07 11 20 59	25 70 14 66 70
23 52 37 83 17	73 20 88 98 37	68 93 59 14 16	26 25 22 96 63	05 52 28 25 62
04 49 35 24 94	75 24 63 38 24	45 86 25 10 25	61 96 27 93 35	65 33 71 24 72
00 54 99 76 54	64 05 18 81 59	96 11 96 38 96	54 69 28 23 91	23 28 72 95 29
35 96 31 53 07	26 89 80 93 45	33 35 13 54 62	77 97 45 00 24	90 10 33 93 33
59 80 80 83 91	45 42 72 68 42	83 60 94 97 00	13 02 12 48 92	78 56 52 01 06
46 05 88 52 36	01 39 09 22 86	77 28 14 40 77	93 91 08 36 47	70 61 74 29 41
32 17 90 05 97	87 37 92 52 41	05 56 70 70 07	86 74 31 71 57	85 39 41 18 38
69 23 46 14 06	20 11 74 52 04	15 95 66 00 00	18 74 39 24 23	97 11 89 63 38
19 56 54 14 30	01 75 87 53 79	40 41 92 15 85	66 67 43 68 06	84 96 28 52 07
45 15 51 49 38	19 47 60 72 46	43 66 79 45 43	59 04 79 00 33	20 82 66 95 41
94 86 43 19 94	36 16 81 08 51	34 88 88 15 53	01 54 03 54 56	05 01 45 11 76
98 08 62 48 26	45 24 02 84 04	44 99 90 88 96	39 09 47 34 07	35 44 13 18 80
33 18 51 62 32	41 94 15 09 49	89 43 54 85 81	88 69 54 19 94	37 54 87 30 43
80 95 10 04 06	96 38 27 07 74	20 15 12 33 87	25 01 62 52 98	94 62 46 11 71
79 75 24 91 40	71 96 12 82 96	69 86 10 25 91	74 85 22 05 39	00 38 75 95 79
18 63 33 25 37	98 14 50 65 71	31 01 02 46 74	05 45 56 14 27	77 93 89 19 36
74 02 94 39 02	77 55 73 22 70	97 79 01 71 19	52 52 75 80 21	80 81 45 17 48
54 17 84 56 11	80 99 33 71 43	05 33 51 29 69	56 12 71 92 55	36 04 09 03 24
11 66 44 98 83	52 07 98 48 27	59 38 17 15 39	09 97 33 34 40	88 46 12 33 56
48 32 47 79 28	31 24 96 47 10	02 29 53 68 70	32 30 75 75 46	15 02 00 99 94
69 07 49 41 38	87 63 79 19 76	35 58 40 44 01	10 51 82 16 15	01 84 87 69 38

2.3.4 Continue in this fashion until all the fish have been randomly assigned to a replicate chamber. In order to fill each replicate chamber with ten fish, the assigned numbers will be used more than once. If a number is read from the table that was not assigned to a replicate chamber, then ignore it and continue to the next number. If a replicate chamber becomes filled and a number is read from the table that corresponds to it, then ignore that value and continue to the next number. The first ten random assignments of fish to replicate chambers for the example are summarized in Table A.3.

TABLE A.3. EXAMPLE OF RANDOM ASSIGNMENT OF FIRST TEN FISH TO REPLICATE CHAMBERS

Fish		Assignment	
First	fish taken from tank	25.0% effluent,	replicate chamber 1
Second	fish taken from tank	6.25% effluent,	replicate chamber 2
Third	fish taken from tank	50.0% effluent,	replicate chamber 4
Fourth	fish taken from tank	100.0% effluent,	replicate chamber 4
Fifth	fish taken from tank	6.25% effluent,	replicate chamber 1
Sixth	fish taken from tank	25.0% effluent,	replicate chamber 4
Seventh	fish taken from tank	50.0% effluent,	replicate chamber 1
Eighth	fish taken from tank	100.0% effluent,	replicate chamber 3
Ninth	fish taken from tank	50.0% effluent,	replicate chamber 2
Tenth	fish taken from tank	100.0% effluent,	replicate chamber 4

2.3.5 Four double-digit numbers were assigned to each replicate chamber (instead of one, two, or three double-digit numbers) in order to make efficient use of the random number table (Table A.2). To illustrate, consider the assignment of only one double-digit number to each replicate chamber: the first column of assigned numbers in Table A.1. Whenever the numbers 00 and 25 through 99 are read from Table A.2, they will be disregarded and the next number will be read.

2.4 RANDOMIZATION OF REPLICATE CHAMBERS TO POSITIONS EXAMPLE

2.4.1 Next consider the random assignment of the 24 replicate chambers to positions within the water bath (or equivalent). Assume that the replicate chambers are to be positioned in a four row by six column rectangular array. The first step is to label the positions in the water bath. Table A.4 provides an example layout.

TABLE A.4 RANDOM ASSIGNMENT OF REPLICATE CHAMBERS TO POSITIONS: EXAMPLE LABELING THE POSITIONS WITHIN THE WATER BATH

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24

2.4.2 The second step is to assign each of the 24 positions four double-digit numbers. An example of this assignment is provided in Table A.5. Note that the double digits 00 and 97 through 99 were not used.

TABLE A.5. RANDOM ASSIGNMENT OF REPLICATE CHAMBERS TO POSITIONS: EXAMPLE ASSIGNED NUMBERS FOR EACH POSITION

Assigned Numbers	Position
01, 25, 49, 73	1
02, 26, 50, 74	2
03, 27, 51, 75	3
04, 28, 52, 76	4
05, 29, 53, 77	5
06, 30, 54, 78	6
07, 31, 55, 79	7
08, 32, 56, 80	8
09, 33, 57, 81	9
10, 34, 58, 82	10
11, 35, 59, 83	11
12, 36, 60, 84	12
13, 37, 61, 85	13
14, 38, 62, 86	14
15, 39, 63, 87	15
16, 40, 64, 88	16
17, 41, 65, 89	17
18, 42, 66, 90	18
19, 43, 67, 91	19
20, 44, 68, 92	20
21, 45, 69, 93	21
22, 46, 70, 94	22
23, 47, 71, 95	23
24, 48, 72, 96	24

2.4.3 The random numbers used to carry out the random assignment of replicate chambers to positions are provided in Table A.2. The third step is to choose a starting position in Table A.2, and read the first double-digit number. The first number read identifies the position for the first replicate chamber of the control. For the example, the first entry in row 10 of Table A.2 was chosen as the starting position. The first number in this row was 73. According to Table A.5, this number corresponds to position 1. Thus, the first replicate chamber for the control will be placed in position 1.

2.4.4 The next step is to read the double-digit number to the right of the first one. The second number identifies the position for the second replicate chamber of the control. Continuing the example, the second number read in row 10 of Table A.2 is 79. According to Table A.5, this number corresponds to position 7. Thus, the second replicate chamber for the control will be placed in position 7.

2.4.5 Continue in this fashion until all the replicate chambers have been assigned to a position. The first four numbers read will identify the positions for the control replicate chambers, the second four numbers read will identify the positions for the lowest effluent concentration replicate chambers, and so on. If a number is read from the table that was not assigned to a position, then ignore that value and continue to the next number. If a number is repeated in Table A.2, then ignore the repeats and continue to the next number. The complete randomization of replicate chambers to positions for the example is displayed in Table A.6.

TABLE A.6. RANDOM ASSIGNMENT OF REPLICATE CHAMBERS TO POSITIONS: EXAMPLE ASSIGNMENT OF ALL 24 POSITIONS

Control	100.0%	6.25%	6.25%	6.25%	12.5%
Control	12.5%	Control	25.0%	12.5%	25.0%
100.0%	50.0%	100.0%	Control	100.0%	25.0%
50.0%	50.0%	25.0%	50.0%	12.5%	6.25%

2.4.6 Four double-digit numbers were assigned to each position (instead of one, two, or three) in order to make efficient use of the random number table (Table A.2). To illustrate, consider the assignment of only one double-digit number to each position: the first column of assigned numbers in Table A.5. Whenever the numbers 00 and 25 through 99 are read from Table A.2, they will be disregarded and the next number will be read.

3. **OUTLIERS**

3.1 An outlier is an inconsistent or questionable data point that appears unrepresentative of the general trend exhibited by the majority of the data. Outliers may be detected by tabulation of the data, plotting, and by an analysis of the residuals. An explanation should be sought for any questionable data points. Without an explanation, data points should be discarded only with extreme caution. If there is no explanation, the analysis should be performed both with and without the outlier, and the results of both analyses should be reported.

3.2 Gentleman-Wilk's A statistic gives a test for the condition that the extreme observation may be considered an outlier. For a discussion of this, and other techniques for evaluating outliers, see Draper and John (1981).

APPENDIX B

VALIDATING NORMALITY AND HOMOGENEITY OF VARIANCE ASSUMPTIONS

1. **INTRODUCTION**

1.1 Dunnett's Procedure and the t test with Bonferroni's adjustment are parametric procedures based on the assumptions that the observations within treatments are independent and normally distributed, and that the variance of the observations is homogeneous across all toxicant concentrations and the control. These assumptions should be checked prior to using these tests, to determine if they have been met. Tests for validating the assumptions are provided in the following discussion. If the tests fail (if the data do not meet the assumptions), a nonparametric procedure such as Steel's Many-one Rank Test may be more appropriate. However, the decision on whether to use parametric or nonparametric tests may be a judgment call, and a statistician should be consulted in selecting the analysis.

2. **TEST FOR NORMAL DISTRIBUTION OF DATA**

2.1 SHAPIRO-WILK'S TEST

2.1.1 One formal test for normality is the Shapiro-Wilk's Test (Conover, 1980). The test statistic is obtained by dividing the square of an appropriate linear combination of the sample order statistics by the usual symmetric estimate of variance. The calculated W must be greater than zero and less than or equal to one. This test is recommended for a sample size of 50 or less. If the sample size is greater than 50, the Kolmogorov "D" statistic (Stephens, 1974) is recommended. An example of the Shapiro-Wilk's test is provided below.

2.2 The example uses growth data from the Fathead Minnow Larval Survival and Growth Test. The same data are used in the discussion of the homogeneity of variance determination in Paragraph 3 and Dunnett's Procedure in Appendix C. The data, the mean and variance of the observations at each concentration, including the control, are listed in Table B.1.

TABLE B.1. FATHEAD LARVAL, *PIMEPHALES PROMELAS*, LARVAL GROWTH DATA (WEIGHT IN MG) FOR THE SHAPIRO-WILK'S TEST

		NaPCP Concentration (µg/L)			
Replicate	Control	32	64	128	256
A	0.711	0.646	0.669	0.629	0.650
B	0.662	0.626	0.669	0.680	0.558
C	0.718	0.723	0.694	0.513	0.606
D	0.767	0.700	0.676	0.672	0.508
Mean($\bar{Y}_i$)	0.714	0.674	0.677	0.624	0.580
S_i^2	0.0018	0.0020	0.0001	0.0059	0.0037
i	1	2	3	4	5

2.3 The first step of the test for normality is to center the observations by subtracting the mean of all the observations within a concentration from each observation in that concentration. The centered observations are listed in Table B.2.

TABLE B.2. EXAMPLE OF SHAPIRO-WILK'S TEST: CENTERED OBSERVATIONS

		NaPCP Concentration (µg/L)			
Replicate	Control	32	64	128	256
A	-0.003	-0.028	-0.008	0.005	0.070
B	-0.052	-0.048	-0.008	0.056	-0.022
C	0.004	0.049	0.017	-0.111	0.026
D	0.053	0.026	-0.001	0.048	-0.072

2.4 Calculate the denominator, D, of the test statistic:

$$D = \sum_{i=1}^{n} (X_i - \overline{X})^2$$

Where: X_i = the centered observations and $\overline{X}$ is the overall mean of the centered observations. For this set of data, $\overline{X}$ = 0, and D = 0.0412.

2.5 Order the centered observations from smallest to largest.

$X^{(1)} \leq X^{(2)} \leq ... \leq X^{(n)}$

where $X^{(i)}$ denotes the ith ordered observation. The ordered observations are listed in Table B.3.

TABLE B.3. EXAMPLE OF THE SHAPIRO-WILK'S TEST: ORDERED OBSERVATIONS

i	$X^{(i)}$	i	$X^{(i)}$
1	-0.111	11	0.004
2	-0.072	12	0.005
3	-0.052	13	0.017
4	-0.048	14	0.026
5	-0.028	15	0.026
6	-0.022	16	0.048
7	-0.008	17	0.049
8	-0.008	18	0.053
9	-0.003	19	0.056
10	-0.001	20	0.070

2.6 From Table B.4, for the number of observations, n, obtain the coefficients a_1, a_2, ..., a_k, where k is n/2 if n is even, and (n-1)/2 if n is odd. For the data in this example, n = 20, k = 10. The a_i values are listed in Table B.5.

TABLE B.4. COEFFICIENTS FOR THE SHAPIRO-WILK'S TEST (Conover, 1980)

i \ n	Number of Observations 2	3	4	5	6	7	8	9	10
1	0.7071	0.7071	0.6872	0.6646	0.6431	0.6233	0.6052	0.5888	0.5739
2	-	0.0000	0.1667	0.2413	0.2806	0.3031	0.3164	0.3244	0.3291
3	-	-	-	0.0000	0.0875	0.1401	0.1743	0.1976	0.2141
4	-	-	-	-	-	0.0000	0.0561	0.0947	0.1224
5	-	-	-	-	-	-	-	0.0000	0.0399

i \ n	Number of Observations 11	12	13	14	15	16	17	18	19	20
1	0.5601	0.5475	0.5359	0.5251	0.5150	0.5056	0.4968	0.4886	0.4808	0.4734
2	0.3315	0.3325	0.3325	0.3318	0.3306	0.3209	0.3273	0.3253	0.3232	0.3211
3	0.2260	0.2347	0.2412	0.2460	0.2495	0.2521	0.2540	0.2553	0.2561	0.2565
4	0.1429	0.1586	0.1707	0.1802	0.1878	0.1939	0.1988	0.2027	0.2059	0.2085
5	0.0695	0.0922	0.1099	0.1240	0.1353	0.1447	0.1524	0.1587	0.1641	0.1686
6	0.0000	0.0303	0.0539	0.0727	0.0880	0.1005	0.1109	0.1197	0.1271	0.1334
7	-	-	0.0000	0.0240	0.0433	0.0593	0.0725	0.0837	0.0932	0.1013
8	-	-	-	-	0.0000	0.0196	0.0359	0.0496	0.0612	0.0711
9	-	-	-	-	-	-	0.0000	0.0163	0.0303	0.0422
10	-	-	-	-	-	-	-	-	0.0000	0.0140

i \ n	Number of Observations 21	22	23	24	25	26	27	28	29	30
1	0.4643	0.4590	0.4542	0.4493	0.4450	0.4407	0.4366	0.4328	0.4291	0.4254
2	0.3185	0.3156	0.3126	0.3098	0.3069	0.3043	0.3018	0.2992	0.2968	0.2944
3	0.2578	0.2571	0.2563	0.2554	0.2543	0.2533	0.2522	0.2510	0.2499	0.2487
4	0.2119	0.2131	0.2139	0.2145	0.2148	0.2151	0.2152	0.2151	0.2150	0.2148
5	0.1736	0.1764	0.1787	0.1807	0.1822	0.1836	0.1848	0.1857	0.1864	0.1870
6	0.1399	0.1443	0.1480	0.1512	0.1539	0.1563	0.1584	0.1601	0.1616	0.1630
7	0.1092	0.1150	0.1201	0.1245	0.1283	0.1316	0.1346	0.1372	0.1395	0.1415
8	0.0804	0.0878	0.0941	0.0997	0.1046	0.1089	0.1128	0.1162	0.1192	0.1219
9	0.0530	0.0618	0.0696	0.0764	0.0923	0.0876	0.0923	0.0965	0.1002	0.1036
10	0.0263	0.0368	0.0459	0.0539	0.0610	0.0672	0.0728	0.0778	0.0822	0.0862
11	0.0000	0.0122	0.0228	0.0321	0.0403	0.0476	0.0540	0.0598	0.0650	0.0697
12	-	-	0.0000	0.0107	0.0200	0.0284	0.0358	0.0424	0.0483	0.0537
13	-	-	-	-	0.0000	0.0094	0.0178	0.0253	0.0320	0.0381
14	-	-	-	-	-	-	0.0000	0.0084	0.0159	0.0227
15	-	-	-	-	-	-	-	-	0.0000	0.0076

TABLE B.4. COEFFICIENTS FOR THE SHAPIRO WILK'S TEST (CONTINUED)

i \ n	Number of Observations 31	32	33	34	35	36	37	38	39	40
1	0.4220	0.4188	0.4156	0.4127	0.4096	0.4068	0.4040	0.4015	0.3989	0.3964
2	0.2921	0.2898	0.2876	0.2854	0.2834	0.2813	0.2794	0.2774	0.2755	0.2737
3	0.2475	0.2462	0.2451	0.2439	0.2427	0.2415	0.2403	0.2391	0.2380	0.2368
4	0.2145	0.2141	0.2137	0.2132	0.2127	0.2121	0.2116	0.2110	0.2104	0.2098
5	0.1874	0.1878	0.1880	0.1882	0.1883	0.1883	0.1883	0.1881	0.1880	0.1878
6	0.1641	0.1651	0.1660	0.1667	0.1673	0.1678	0.1663	0.1686	0.1689	0.1691
7	0.1433	0.1449	0.1463	0.1475	0.1487	0.1496	0.1505	0.1513	0.1520	0.1526
8	0.1243	0.1265	0.1284	0.1301	0.1317	0.1331	0.1344	0.1356	0.1366	0.1376
9	0.1066	0.1093	0.1118	0.1140	0.1160	0.1179	0.1196	0.1211	0.1225	0.1237
10	0.0899	0.0931	0.0961	0.0988	0.1013	0.1036	0.1056	0.1075	0.1092	0.1108
11	0.0739	0.0777	0.0812	0.0844	0.0873	0.0900	0.0924	0.0947	0.0967	0.0986
12	0.0585	0.0629	0.0669	0.0706	0.0739	0.0770	0.0798	0.0824	0.0848	0.0870
13	0.0435	0.0485	0.0530	0.0572	0.0610	0.0645	0.0677	0.0706	0.0733	0.0759
14	0.0289	0.0344	0.0395	0.0441	0.0484	0.0523	0.0559	0.0592	0.0622	0.0651
15	0.0144	0.0206	0.0262	0.0314	0.0361	0.0404	0.0444	0.0481	0.0515	0.0546
16	0.0000	0.0068	0.0131	0.0187	0.0239	0.0287	0.0331	0.0372	0.0409	0.0444
17	-	-	0.0000	0.0062	0.0119	0.0172	0.0220	0.0264	0.0305	0.0343
18	-	-	-	-	0.0000	0.0057	0.0110	0.0158	0.0203	0.0244
19	-	-	-	-	-	-	0.0000	0.0053	0.0101	0.0146
20	-	-	-	-	-	-	-	-	0.0000	0.0049

i \ n	Number of Observations 41	42	43	44	45	46	47	48	49	50
1	0.3940	0.3917	0.3894	0.3872	0.3850	0.3830	0.3808	0.3789	0.3770	0.3751
2	0.2719	0.2701	0.2684	0.2667	0.2651	0.2635	0.2620	0.2604	0.2589	0.2574
3	0.2357	0.2345	0.2334	0.2323	0.2313	0.2302	0.2291	0.2281	0.2271	0.2260
4	0.2091	0.2085	0.2078	0.2072	0.2065	0.2058	0.2052	0.2045	0.2038	0.2032
5	0.1876	0.1874	0.1871	0.1868	0.1865	0.1862	0.1859	0.1855	0.1851	0.1847
6	0.1693	0.1694	0.1695	0.1695	0.1695	0.1695	0.1695	0.1693	0.1692	0.1691
7	0.1531	0.1535	0.1539	0.1542	0.1545	0.1548	0.1550	0.1551	0.1553	0.1554
8	0.1384	0.1392	0.1398	0.1405	0.1410	0.1415	0.1420	0.1423	0.1427	0.1430
9	0.1249	0.1259	0.1269	9,1278	0.1286	0.1293	0.1300	0.1306	0.1312	0.1317
10	0.1123	0.1136	0.1149	0.1160	9.1170	0.1180	0.1189	0.1197	0.1205	0.1212
11	0.1004	0.1020	0.1035	0.1049	0.1062	0.1073	0.1085	0.1095	0.1105	0.1113
12	0.0891	0.0909	0.0927	0.0943	0.0959	0.0972	0.0986	0.0998	0.1010	0.1020
13	0.0782	0.0804	0.0824	0.0842	0.0860	0.0876	0.0892	0.0906	0.0919	0.0932
14	0.0677	0.0701	0.0724	0.0745	0.0765	0.0783	0.0801	0.0817	0.0832	0.0846
15	0.0575	0.0602	0.0628	0.0651	0.0673	0.0694	0.0713	0.0731	0.0748	0.0764
16	0.0476	0.0506	0.0534	0.0560	0.0584	0.0607	0.0628	0.0648	0.0667	0.0685
17	0.0379	0.0411	0.0442	0.0471	0.0497	0.0522	0.0546	0.0568	0.0588	0.0608
18	0.0283	0.0318	0.0352	0.0383	0.0412	0.0439	0.0465	0.0489	0.0511	0.0532
19	0.0188	0.0227	0.0263	0.0296	0.0328	0.0357	0.0385	0.0411	0.0436	0.0459
20	0.0094	0.0136	0.0175	0.0211	0.0245	0.0277	0.0307	0.0335	0.0361	0.0386
21	0.0000	0.0045	0.0087	0.0126	0.0163	0.0197	0.0229	0.0259	0.0288	0.0314
22	-	-	0.0000	0.0042	0.0081	0.0118	0.0153	0.0185	0.0215	0.0244
23	-	-	-	-	0.0000	0.0039	0.0076	0.0111	0.0143	0.0174
24	-	-	-	-	-	-	0.0000	0.0037	0.0071	0.0104
25	-	-	-	-	-	-	-	-	0.0000	0.0035

2.7 Compute the test statistic, W, as follows:

$$W = \frac{1}{D} \left[\sum_{i=1}^{k} a_i \left(X^{(n-i+1)} - X^{(i)}\right)\right]^2$$

The differences, $X^{(n-i+1)} - X^{(i)}$, are listed in Table B.5.

2.8 The decision rule for this test is to compare the critical value from Table B.6 to the computed W. If the computed value is less than the critical value, conclude that the data are not normally distributed. For this example, the critical value at a significance level of 0.01 and 20 observations (n) is 0.868. The calculated value, 0.959, is not less than the critical value. Therefore, conclude that the data are normally distributed.

TABLE B.5. EXAMPLE OF THE SHAPIRO-WILK'S TEST: TABLE OF COEFFICIENTS AND DIFFERENCES

i	a_i	$X^{(n-i+1)} - X^{(i)}$	
1	0.4734	0.181	$X^{(20)} - X^{(1)}$
2	0.3211	0.128	$X^{(19)} - X^{(2)}$
3	0.2565	0.105	$X^{(18)} - X^{(3)}$
4	0.2085	0.097	$X^{(17)} - X^{(4)}$
5	0.1686	0.076	$X^{(16)} - X^{(5)}$
6	0.1334	0.048	$X^{(15)} - X^{(6)}$
7	0.1013	0.034	$X^{(14)} - X^{(7)}$
8	0.0711	0.025	$X^{(13)} - X^{(8)}$
9	0.0422	0.008	$X^{(12)} - X^{(9)}$
10	0.0140	0.005	$X^{(11)} - X^{(10)}$

2.9 In general, if the data fail the test for normality, a transformation such as to log values may normalize the data. After transforming the data, repeat the Shapiro-Wilk's Test for normality.

2.10 KOLMOGOROV "D" TEST

2.10.1 A formal two-sided test for normality is the Kolmogorov "D" Test. The test statistic is calculated by obtaining the difference between the cumulative distribution function estimated from the data and the standard normal cumulative distribution function for each standardized observation. This test is recommended for a sample size greater than 50. If the sample size is less than or equal to 50, then the Shapiro Wilk's Test is recommended. An example of the Kolmogorov "D" test is provided below.

2.10.2 The example uses reproduction data from the daphnid, *Ceriodaphnia dubia,* Survival and Reproduction Test. The observed data and the mean of the observations at each concentration, including the control, are listed in Table B.7.

2.10.3 The first step of the test for normality is to center the observations by subtracting the mean of all the observations within a concentration from each observation in that concentration. The centered observations for the example are listed in Table B.8.

TABLE B.6. QUANTILES OF THE SHAPIRO-WILK'S TEST STATISTIC (Conover, 1980)

n	0.01	0.02	0.05	0.10	0.50	0.90	0.95	0.98	0.99
3	0.753	0.756	0.767	0.789	0.959	0.998	0.999	1.000	1.000
4	0.687	0.707	0.748	0.792	0.935	0.987	0.992	0.996	0.997
5	0.686	0.715	0.762	0.806	0.927	0.979	0.986	0.991	0.993
6	0.713	0.743	0.788	0.826	0.927	0.974	0.981	0.986	0.989
7	0.730	0.760	0.803	0.838	0.928	0.972	0.979	0.985	0.988
8	0.749	0.778	0.818	0.851	0.932	0.972	0.978	0.984	0.987
9	0.764	0.791	0.829	0.859	0.935	0.972	0.978	0.984	0.986
10	0.781	0.806	0.842	0.869	0.938	0.972	0.978	0.983	0.986
11	0.792	0.817	0.850	0.876	0.940	0.973	0.979	0.984	0.986
12	0.805	0.828	0.859	0.883	0.943	0.973	0.979	0.984	0.986
13	0.814	0.837	0.866	0.889	0.945	0.974	0.979	0.984	0.986
14	0.825	0.846	0.874	0.895	0.947	0.975	0.980	0.984	0.986
15	0.835	0.855	0.881	0.901	0.950	0.975	0.980	0.984	0.987
16	0.844	0.863	0.887	0.906	0.952	0.976	0.981	0.985	0.987
17	0.851	0.869	0.892	0.910	0.954	0.977	0.981	0.985	0.987
18	0.858	0.874	0.897	0.914	0.956	0.978	0.982	0.986	0.988
19	0.863	0.879	0.901	0.917	0.957	0.978	0.982	0.986	0.988
20	0.868	0.884	0.905	0.920	0.959	0.979	0.983	0.986	0.988
21	0.873	0.888	0.908	0.923	0.960	0.980	0.983	0.987	0.989
22	0.878	0.892	0.911	0.926	0.961	0.980	0.984	0.987	0.989
23	0.881	0.895	0.914	0.928	0.962	0.981	0.984	0.987	0.989
24	0.884	0.898	0.916	0.930	0.963	0.981	0.984	0.987	0.989
25	0.888	0.901	0.918	0.931	0.964	0.981	0.985	0.988	0.989
26	0.891	0.904	0.920	0.933	0.965	0.982	0.985	0.988	0.989
27	0.894	0.906	0.923	0.935	0.965	0.982	0.985	0.988	0.990
28	0.896	0.908	0.924	0.936	0.966	0.982	0.985	0.988	0.990
29	0.898	0.910	0.926	0.937	0.966	0.982	0.985	0.988	0.990
30	0.900	0.912	0.927	0.939	0.967	0.983	0.985	0.988	0.990
31	0.902	0.914	0.929	0.940	0.967	0.983	0.986	0.988	0.990
32	0.904	0.915	0.930	0.941	0.968	0.983	0.986	0.988	0.990
33	0.906	0.917	0.931	0.942	0.968	0.983	0.986	0.989	0.990
34	0.908	0.919	0.933	0.943	0.969	0.983	0.986	0.989	0.990
35	0.910	0.920	0.934	0.944	0.969	0.984	0.986	0.989	0.990
36	0.912	0.922	0.935	0.945	0.970	0.984	0.986	0.989	0.990
37	0.914	0.924	0.936	0.946	0.970	0.984	0.987	0.989	0.990
38	0.916	0.925	0.938	0.947	0.971	0.984	0.987	0.989	0.990
39	0.917	0.927	0.939	0.948	0.971	0.984	0.987	0.989	0.991
40	0.919	0.928	0.940	0.949	0.972	0.985	0.987	0.989	0.991
41	0.920	0.929	0.941	0.950	0.972	0.985	0.987	0.989	0.991
42	0.922	0.930	0.942	0.951	0.972	0.985	0.987	0.989	0.991
43	0.923	0.932	0.943	0.951	0.973	0.985	0.987	0.990	0.991
44	0.924	0.933	0.944	0.952	0.973	0.985	0.987	0.990	0.991
45	0.926	0.934	0.945	0.953	0.973	0.985	0.988	0.990	0.991
46	0.927	0.935	0.945	0.953	0.974	0.985	0.988	0.990	0.991
47	0.928	0.936	0.946	0.954	0.974	0.985	0.988	0.990	0.991
48	0.929	0.937	0.947	0.954	0.974	0.985	0.988	0.990	0.991
49	0.929	0.937	0.947	0.955	0.974	0.985	0.988	0.990	0.991
50	0.930	0.938	0.947	0.955	0.974	0.985	0.988	0.990	0.991

TABLE B.7. *CERIODAPHNIA DUBIA* REPRODUCTION DATA FOR THE KOLMOGOROV "D" TEST

	Effluent Concentration (%)					
Replicate	Control	1.56	3.12	6.25	12.5	25.0
1	27	32	39	27	19	10
2	30	35	30	34	25	13
3	29	32	33	36	26	7
4	31	26	33	34	17	7
5	16	18	36	31	16	7
6	15	29	33	27	21	10
7	18	27	33	33	23	10
8	17	16	27	31	15	16
9	14	35	38	33	18	12
10	27	13	44	31	10	2
Mean	22.4	26.3	34.6	31.7	19.0	9.4

TABLE B.8. CENTERED OBSERVATIONS FOR KOLMOGOROV “D” EXAMPLE

	Effluent Concentration (%)					
Replicate	Control	1.56	3.12	6.25	12.5	25.0
1	4.6	5.7	4.4	-4.7	0.0	0.6
2	7.6	8.7	-4.6	2.3	6.0	3.6
3	6.6	5.7	-1.6	4.3	7.0	-2.4
4	8.6	-0.3	-1.6	2.3	-2.0	-2.4
5	-6.4	-8.3	1.4	-0.7	-3.0	-2.4
6	-7.4	2.7	-1.6	-4.7	2.0	0.6
7	-4.4	0.7	-1.6	1.3	4.0	0.6
8	-5.4	-10.3	-7.6	-0.7	-4.0	6.6
9	-8.4	8.7	3.4	1.3	-1.0	2.6
10	4.6	-13.3	9.4	-0.7	-9.0	-7.4

2.10.4 Order the centered observations from smallest to largest:

$$X^{(1)} \leq X^{(2)} \leq ... \leq X^{(n)}$$

where $X^{(i)}$ denotes the ith ordered observation, and n denotes the total number of centered observations. The ordered observations for the example are listed in Table B.9.

TABLE B.9. EXAMPLE CALCULATION OF THE KOLMOGOROV "D" STATISTIC

i	$X^{(i)}$	z_i	p_i	D_i+	D_i-
1	-13.3	-2.51	0.0060	0.0107	0.0060
2	-10.3	-1.94	0.0262	0.0071	0.0095
3	-9.0	-1.70	0.0446	0.0054	0.0113
4	-8.4	-1.58	0.0571	0.0096	0.0071
5	-8.3	-1.57	0.0582	0.0251	-0.0085
6	-7.6	-1.43	0.0764	0.0236	-0.0069
7	-7.4	-1.40	0.0808	0.0359	-0.0192
8	-7.4	-1.40	0.0808	0.0525	-0.0359
9	-6.4	-1.21	0.1131	0.0369	-0.0202
10	-5.4	-1.02	0.1539	0.0128	0.0039
11	-4.7	-0.89	0.1867	-0.0034	0.0200
12	-4.7	-0.89	0.1867	0.0133	0.0034
13	-4.6	-0.87	0.1922	0.0245	-0.0078
14	-4.4	-0.83	0.2033	0.0300	-0.0134
15	-4.0	-0.75	0.2266	0.0234	-0.0067
16	-3.0	-0.57	0.2843	-0.0176	0.0343
17	-2.4	-0.45	0.3264	-0.0431	0.0597
18	-2.4	-0.45	0.3264	-0.0264	0.0431
19	-2.4	-0.45	0.3264	-0.0097	0.0264
20	-2.0	-0.38	0.3520	-0.0187	0.0353
21	-1.6	-0.30	0.3821	-0.0321	0.0488
22	-1.6	-0.30	0.3821	-0.0154	0.0321
23	-1.6	-0.30	0.3821	0.0012	0.0154
24	-1.6	-0.30	0.3821	0.0179	-0.0012
25	-1.0	-0.19	0.4247	-0.0080	0.0247
26	-0.7	-0.13	0.4483	-0.0150	0.0316
27	-0.7	-0.13	0.4483	0.0017	0.0150
28	-0.7	-0.13	0.4483	0.0184	-0.0017
29	-0.3	-0.06	0.4761	0.0072	0.0094
30	0.0	0.00	0.5000	0.0000	0.0167
31	0.6	0.11	0.5438	-0.0271	0.0438
32	0.6	0.11	0.5438	-0.0105	0.0271
33	0.6	0.11	0.5438	0.0062	0.0105
34	0.7	0.13	0.5517	0.0150	0.0017
35	1.3	0.25	0.5987	-0.0154	0.0320
36	1.3	0.25	0.5987	0.0013	0.0154
37	1.4	0.26	0.6026	0.0141	0.0026
38	2.0	0.38	0.6480	-0.0147	0.0313
39	2.3	0.43	0.6664	-0.0164	0.0331
40	2.3	0.43	0.6664	0.0003	0.0164
41	2.6	0.49	0.6879	-0.0046	0.0212
42	2.7	0.51	0.6950	0.0050	0.0117
43	3.4	0.64	0.7389	-0.0222	0.0389
44	3.6	0.68	0.7517	-0.0184	0.0350
45	4.0	0.75	0.7734	-0.0234	0.0401
46	4.3	0.81	0.7910	-0.0243	0.0410
47	4.4	0.83	0.7967	-0.0134	0.0300

TABLE B.9. EXAMPLE CALCULATION OF THE KOLMOGOROV "D" STATISTIC (CONTINUED)

i	$X^{(i)}$	z_i	p_i	D_i+	D_i-
48	4.6	0.87	0.8078	-0.0078	0.0245
49	4.6	0.87	0.8078	0.0089	0.0078
50	5.7	1.08	0.8599	-0.0266	0.0432
51	5.7	1.08	0.8599	-0.0099	0.0266
52	6.0	1.13	0.8708	-0.0041	0.0208
53	6.6	1.25	0.8944	-0.0111	0.0277
54	6.6	1.25	0.8944	0.0056	0.0111
55	7.0	1.32	0.9066	0.0101	0.0066
56	7.6	1.43	0.9236	0.0097	0.0069
57	8.6	1.62	0.9474	0.0026	0.0141
58	8.7	1.64	0.9495	0.0172	-0.0005
59	8.7	1.64	0.9495	0.0338	-0.0172
60	9.4	1.77	0.9616	0.0384	-0.0217

2.10.5 The next step is to standardize the ordered observations. Let z_i denote the standardized value of the ith ordered observation. Then,

$$z_i = \frac{X^{(i)}}{s} \ \ and \ \ s^2 = \frac{\Sigma[X^{(i)}]^2}{(n-1)}$$

For the example, s = 5.3, and the standardized observations are listed in Table B.9.

2.10.6 From Table B.10, obtain the value of the standard normal cumulative distribution function (standard normal CDF) at z_i. Denote this value as p_i. Note that negative z are not listed in Table B.10. The value of the standard normal CDF at a negative number is one minus the value of the standard normal CDF at the absolute value of that number. For example, since the value of the standard normal CDF at 3.21 is 0.9993, the value of the standard normal CDF at -3.21 is 1 - 0.9993 = 0.0007. The p_i values for the example data are listed in Table B.9.

TABLE B.10. P IS THE VALUE OF THE STANDARD NORMAL CUMULATIVE DISTRIBUTION AT Z

z	p	z	p	z	p	z	p
0.00	0.5000	0.41	0.6591	0.82	0.7939	1.23	0.8907
0.01	0.5040	0.42	0.6628	0.83	0.7967	1.24	0.8925
0.02	0.5080	0.43	0.6664	0.84	0.7995	1.25	0.8944
0.03	0.5120	0.44	0.6700	0.85	0.8023	1.26	0.8962
0.04	0.5160	0.45	0.6736	0.86	0.8051	1.27	0.8980
0.05	0.5199	0.46	0.6772	0.87	0.8078	1.28	0.8997
0.06	0.5239	0.47	0.6808	0.88	0.8106	1.29	0.9015
0.07	0.5279	0.48	0.6844	0.89	0.8133	1.30	0.9032
0.08	0.5319	0.49	0.6879	0.90	0.8159	1.31	0.9049
0.09	0.5359	0.50	0.6915	0.91	0.8186	1.32	0.9066
0.10	0.5398	0.51	0.6950	0.92	0.8212	1.33	0.9082
0.11	0.5438	0.52	0.6985	0.93	0.8238	1.34	0.9099
0.12	0.5478	0.53	0.7019	0.94	0.8264	1.35	0.9115
0.13	0.5517	0.54	0.7054	0.95	0.8289	1.36	0.9131
0.14	0.5557	0.55	0.7088	0.96	0.8315	1.37	0.9147
0.15	0.5596	0.56	0.7123	0.97	0.8340	1.38	0.9162
0.16	0.5636	0.57	0.7157	0.98	0.8365	1.39	0.9177
0.17	0.5675	0.58	0.7190	0.99	0.8389	1.40	0.9192
0.18	0.5714	0.59	0.7224	1.00	0.8413	1.41	0.9207
0.19	0.5753	0.60	0.7257	1.01	0.8438	1.42	0.9222
0.20	0.5793	0.61	0.7291	1.02	0.8461	1.43	0.9236
0.21	0.5832	0.62	0.7324	1.03	0.8485	1.44	0.9251
0.22	0.5871	0.63	0.7357	1.04	0.8508	1.45	0.9265
0.23	0.5910	0.64	0.7389	1.05	0.8531	1.46	0.9279
0.24	0.5948	0.65	0.7422	1.06	0.8554	1.47	0.9292
0.25	0.5987	0.66	0.7454	1.07	0.8577	1.48	0.9306
0.26	0.6026	0.67	0.7486	1.08	0.8599	1.49	0.9319
0.27	0.6064	0.68	0.7517	1.09	0.8621	1.50	0.9332
0.28	0.6103	0.69	0.7549	1.10	0.8643	1.51	0.9345
0.29	0.6141	0.70	0.7580	1.11	0.8665	1.52	0.9357
0.30	0.6179	0.71	0.7611	1.12	0.8686	1.53	0.9370
0.31	0.6217	0.72	0.7642	1.13	0.8708	1.54	0.9382
0.32	0.6255	0.73	0.7673	1.14	0.8729	1.55	0.9394
0.33	0.6293	0.74	0.7704	1.15	0.8749	1.56	0.9406
0.34	0.6331	0.75	0.7734	1.16	0.8770	1.57	0.9418
0.35	0.6368	0.76	0.7764	1.17	0.8790	1.58	0.9429
0.36	0.6406	0.77	0.7794	1.18	0.8810	1.59	0.9441
0.37	0.6443	0.78	0.7823	1.19	0.8830	1.60	0.9452
0.38	0.6480	0.79	0.7852	1.20	0.8849	1.61	0.9463
0.39	0.6517	0.80	0.7881	1.21	0.8869	1.62	0.9474
0.40	0.6554	0.81	0.7910	1.22	0.8888	1.63	0.9484

TABLE B.10. P IS THE VALUE OF THE STANDARD NORMAL CUMULATIVE DISTRIBUTION AT Z (CONTINUED)

z	p	z	p	z	p	z	p
1.64	0.9495	2.05	0.9798	2.46	0.9931	2.87	0.9979
1.65	0.9505	2.06	0.9803	2.47	0.9932	2.88	0.9980
1.66	0.9515	2.07	0.9808	2.48	0.9934	2.89	0.9981
1.67	0.9525	2.08	0.9812	2.49	0.9936	2.90	0.9981
1.68	0.9535	2.09	0.9817	2.50	0.9938	2.91	0.9982
1.69	0.9545	2.10	0.9821	2.51	0.9940	2.92	0.9982
1.70	0.9554	2.11	0.9826	2.52	0.9941	2.93	0.9983
1.71	0.9564	2.12	0.9830	2.53	0.9943	2.94	0.9984
1.72	0.9573	2.13	0.9834	2.54	0.9945	2.95	0.9984
1.73	0.9582	2.14	0.9838	2.55	0.9946	2.96	0.9985
1.74	0.9591	2.15	0.9842	2.56	0.9948	2.97	0.9985
1.75	0.9599	2.16	0.9846	2.57	0.9949	2.98	0.9986
1.76	0.9608	2.17	0.9850	2.58	0.9951	2.99	0.9986
1.77	0.9616	2.18	0.9854	2.59	0.9952	3.00	0.9987
1.78	0.9625	2.19	0.9857	2.60	0.9953	3.01	0.9987
1.79	0.9633	2.20	0.9861	2.61	0.9955	3.02	0.9987
1.80	0.9641	2.21	0.9864	2.62	0.9956	3.03	0.9988
1.81	0.9649	2.22	0.9868	2.63	0.9957	3.04	0.9988
1.82	0.9656	2.23	0.9871	2.64	0.9959	3.05	0.9989
1.83	0.9664	2.24	0.9875	2.65	0.9960	3.06	0.9989
1.84	0.9671	2.25	0.9878	2.66	0.9961	3.07	0.9989
1.85	0.9678	2.26	0.9881	2.67	0.9962	3.08	0.9990
1.86	0.9686	2.27	0.9884	2.68	0.9963	3.09	0.9990
1.87	0.9693	2.28	0.9887	2.69	0.9964	3.10	0.9990
1.88	0.9699	2.29	0.9890	2.70	0.9965	3.11	0.9991
1.89	0.9706	2.30	0.9893	2.71	0.9966	3.12	0.9991
1.90	0.9713	2.31	0.9896	2.72	0.9967	3.13	0.9991
1.91	0.9719	2.32	0.9898	2.73	0.9968	3.14	0.9992
1.92	0.9726	2.33	0.9901	2.74	0.9969	3.15	0.9992
1.93	0.9732	2.34	0.9904	2.75	0.9970	3.16	0.9992
1.94	0.9738	2.35	0.9906	2.76	0.9971	3.17	0.9992
1.95	0.9744	2.36	0.9909	2.77	0.9972	3.18	0.9993
1.96	0.9750	2.37	0.9911	2.78	0.9973	3.19	0.9993
1.97	0.9756	2.38	0.9913	2.79	0.9974	3.20	0.9993
1.98	0.9761	2.39	0.9916	2.80	0.9974	3.21	0.9993
1.99	0.9767	2.40	0.9918	2.81	0.9975	3.22	0.9994
2.00	0.9772	2.41	0.9920	2.82	0.9976	3.23	0.9994
2.01	0.9778	2.42	0.9922	2.83	0.9977	3.24	0.9994
2.02	0.9783	2.43	0.9925	2.84	0.9977	3.25	0.9994
2.03	0.9788	2.44	0.9927	2.85	0.9978	3.26	0.9994
2.04	0.9793	2.45	0.9929	2.86	0.9979	3.27	0.9995

TABLE B.10. P IS THE VALUE OF THE STANDARD NORMAL CUMULATIVE DISTRIBUTION AT Z (CONTINUED)

z	p	z	p	z	p	z	p
3.28	0.9995	3.46	0.9997	3.64	0.9999	3.82	0.9999
3.29	0.9995	3.47	0.9997	3.65	0.9999	3.83	0.9999
3.30	0.9995	3.48	0.0997	3.66	0.9999	3.84	0.9999
3.31	0.9995	3.49	0.9998	3.67	0.9999	3.85	0.9999
3.32	0.9995	3.50	0.9998	3.68	0.9999	3.86	0.9999
3.33	0.9996	3.51	0.9998	3.69	0.9999	3.87	0.9999
3.34	0.9996	3.52	0.9998	3.70	0.9999	3.88	0.9999
3.35	0.9996	3.53	0.9998	3.71	0.9999	3.89	0.9999
3.36	0.9996	3.54	0.9998	3.72	0.9999	3.90	1.0000
3.37	0.9996	3.55	0.9998	3.73	0.9999	3.91	1.0000
3.38	0.9996	3.56	0.9998	3.74	0.9999	3.92	1.0000
3.39	0.9997	3.57	0.9998	3.75	0.9999	3.93	1.0000
3.40	0.9997	3.58	0.9998	3.76	0.9999	3.94	1.0000
3.41	0.9997	3.59	0.9998	3.77	0.9999	3.95	1.0000
3.42	0.9997	3.60	0.9998	3.78	0.9999	3.96	1.0000
3.43	0.9997	3.61	0.9998	3.79	0.9999	3.97	1.0000
3.44	0.9997	3.62	0.9999	3.80	0.9999	3.98	1.0000
3.45	0.9997	3.63	0.9999	3.81	0.9999	3.99	1.0000

2.10.7 Next, calculate the following differences for each ordered observation:

$$D_i+ = (i/n) - p_i$$

$$D_i- = p_i - [(i-1)/n]$$

The differences for the example are listed in Table B.9.

2.10.8 Obtain the maximum of the D_i+, and denote it as D+. Obtain the maximum of the D_i-, and denote it as D-. For the example, D+ = 0.0525, and D- = 0.0597.

2.10.9 Next, obtain the maximum of D+ and D-, and denote it as D. For the example, D = 0.0597.

2.10.10 The test statistic, D*, is calculated as follows:

$$D^* = D(\sqrt{n} - 0.01 + \frac{0.85}{\sqrt{n}})$$

For the example, D* = 0.4684.

2.10.11 The decision rule for the two tailed test is to compare the critical value from Table B.11 to the computed D*. If the computed value is greater than the critical value, conclude that the data are not normally distributed. For this example, the critical value at a significance level of 0.01 is 1.035. The calculated value, 0.4684, is not greater than the critical value. Thus, the conclusion of the test is that the data are normally distributed.

2.10.12 In general, if the data fail the test for normality, a transformation such as the log transformation may normalize the data. After transforming the data, repeat the Kolmogorov "D" test for normality.

TABLE B.11. CRITICAL VALUES FOR THE KOLMOGOROV "D" TEST

Alpha Level	Critical Value
0.010	1.035
0.025	0.955
0.050	0.895
0.100	0.819
0.150	0.775

3. TEST FOR HOMOGENEITY OF VARIANCE

3.1 For Dunnett's Procedure and the t test with Bonferroni's adjustment, the variances of the data obtained from each toxicant concentration and the control are assumed to be equal. Bartlett's Test is a formal test of this assumption. In using this test, it is assumed that the data are normally distributed.

3.2 The data used in this example are growth data from a Fathead Minnow Larval Survival and Growth Test, and are the same data used in Appendices C and D. These data are listed in Table B.12, together with the calculated variance for the control and each toxicant concentration.

TABLE B.12. FATHEAD LARVAL GROWTH DATA (WEIGHT IN MG) USED FOR BARTLETT'S TEST FOR HOMOGENEITY OF VARIANCE

		NaPCP Concentration (µg/L)			
Replicate	Control	32	64	128	256
A	0.711	0.646	0.669	0.629	0.650
B	0.662	0.626	0.669	0.680	0.558
C	0.718	0.723	0.694	0.513	0.606
D	0.767	0.700	0.676	0.672	0.508
Mean($\bar{Y}_i$)	0.714	0.674	0.677	0.624	0.580
S_i^2	0.0018	0.0020	0.0001	0.0059	0.0037
I	1	2	3	4	5

3.3 The test statistic for Bartlett's Test (Snedecor and Cochran, 1980) is as follows:

$$B = \frac{[(\sum_{i=1}^{P} V_i)\ln \bar{S}^2 - \sum_{i=1}^{P} V_i \ln S_i^2]}{C}$$

Where: V_i = degrees of freedom for each toxicant concentration and control p = number of levels of toxicant concentration including the control

ln = $\log_e$

i = 1, 2, ..., p where p is the number of concentrations

n_i = the number of replicates for concentration i.

$$\bar{S}^2 = \frac{(\sum_{i=1}^{P} V_i S_i^2)}{\sum_{i=1}^{P} V_i}$$

$$C = 1 + [3(p-1)]^{-1} [\sum_{i=1}^{P} \frac{1}{V_i} - (\sum_{i=1}^{P} V_i)^{-1}]$$

3.4 Since B is approximately distributed as chi-square with p - 1 degrees of freedom when the variances are equal, the appropriate critical value is obtained from a table of the chi-square distribution for p - 1 degrees of freedom and a significance level of 0.01. If B is less than the critical value then the variances are assumed to be equal.

3.5 For the data in this example, $V_i = 3$, $p = 5$, $\bar{S}^2 = 0.0027$, and $C = 1.133$. The calculated B value is:

$$B = \frac{(15)[\ln(0.0027)] - 3\sum_{i=1}^{P} \ln(S_i^2)}{1.133}$$

$$= \frac{15(-5.9145) - 3(-32.4771)}{1.133}$$

$$= 7.691$$

3.6 Since B is approximately distributed as chi-square with p - 1 degrees of freedom when the variances are equal, the appropriate critical value for the test is 13.277 for a significance level of 0.01. Since B = 7.691 is less than the critical value of 13.277, conclude that the variances are not different.

4. **TRANSFORMATIONS OF THE DATA**

4.1 When the assumptions of normality and/or homogeneity of variance are not met, transformations of the data may remedy the problem, so that the data can be analyzed by parametric procedures, rather than by nonparametric technique such as Steel's Many-one Rank Test or Wilcoxon's Rank Sum Test. Examples of transformations include log, square root, arc sine square root, and reciprocals. After the data have been transformed, Shapiro-Wilk's and Bartlett's tests should be performed on the transformed observations to determine whether the assumptions of normality and/or homogeneity of variance are met.

4.2 ARC SINE SQUARE ROOT TRANSFORMATION (USEPA, 1993)

4.2.1 For data consisting of proportions from a binomial (response/no response; live/dead) response variable, the variance within the ith treatment is proportional to P_i (1 - P_i), where P_i is the expected proportion for the treatment. This clearly violates the homogeneity of variance assumption required by parametric procedures such as Dunnett's Procedure or the t test with Bonferroni's adjustment, since the existence of a treatment effect implies different values of P_i for different treatments, i. Also, when the observed proportions are based on small samples, or when P_i is close to zero or one, the normality assumption may be invalid. The arc sine square root (arc sine$\sqrt{\mathrm{P}}$) transformation is commonly used for such data to stabilize the variance and satisfy the normality requirement.

4.2.2 Arc sine transformation consists of determining the angle (in radians) represented by a sine value. In the case of arc sine square root transformation of mortality data, the proportion of dead (or affected) organisms is taken as the sine value, the square root of the sine value is calculated, and the angle (in radians) for the square root of the sine value is determined. Whenever the proportion dead is 0 or 1, a special modification of the arc sine square root transformation must be used (Bartlett, 1937). An explanation of the arc sine square root transformation and the modification is provided below.

4.2.3 Calculate the response proportion (RP) at each effluent concentration, where:

RP = (number of surviving or "unaffected" organisms)/(number exposed)

<u>Example:</u> If 12 of 20 animals in a given treatment replicate survive:

RP = 12/20

= 0.60

4.2.4 Transform each RP to its arc sine square root, as follows:

4.2.4.1 For RPs greater than zero or less than one:

Angle (radians) = arc sine$\sqrt{\mathrm{RP}}$

<u>Example:</u> If RP = 0.60:

Angle = arc sine$\sqrt{0.60}$

= arc sine 0.7746

= 0.8861 radians

4.2.4.2 Modification of the arc sine square root when RP = 0:

Angle (in radians) = arc sine $\sqrt{1/4\,N}$

Where: N = Number of animals/treatment replicate

Example: If 20 animals are used:

$$\text{Angle} = \text{arc sine } \sqrt{1/80}$$

$$= \text{arc sine } 0.1118$$

$$= 0.1120 \text{ radians}$$

4.2.4.3 Modification of the arc sine square root when RP = 1.0:

Angle = 1.5708 radians - (radians for RP = 0)

Example: Using above value:

$$\text{Angle} = 1.5708 - 0.1120$$

$$= 1.4588 \text{ radians}$$

APPENDIX C

DUNNETT'S PROCEDURE

1. MANUAL CALCULATIONS

1.1 Dunnett's Procedure (Dunnett, 1955; Dunnett, 1964) is used to compare each concentration mean with the control mean to decide if any of the concentrations differ from the control. This test has an overall error rate of alpha, which accounts for the multiple comparisons with the control. It is based on the assumptions that the observations are independent and normally distributed and that the variance of the observations is homogeneous across all concentrations and control (see Appendix B for a discussion on validating the assumptions). Dunnett's Procedure uses a pooled estimate of the variance, which is equal to the error value calculated in an analysis of variance. Dunnett's Procedure can only be used when the same number of replicate test vessels have been used at each concentration and the control. When this condition is not met, a t test with Bonferroni's adjustment is used (see Appendix D).

1.2 The data used in this example are growth data from a Fathead Minnow Larval Survival and Growth Test, and are the same data used in Appendices B and D. These data are listed in Table C.1.

TABLE C.1. FATHEAD MINNOW, *PIMEPHALES PROMELAS,* LARVAL GROWTH DATA (WEIGHT IN MG) USED FOR DUNNETT'S PROCEDURE

		NaPCP Concentration (µg/L)			
Replicate	Control	32	64	128	256
A	0.711	0.517	0.602	0.566	0.455
B	0.662	0.501	0.669	0.612	0.502
C	0.646	0.723	0.694	0.410	0.606
D	0.690	0.560	0.676	0.672	0.254
Mean($\bar{Y}_i$)	0.677	0.575	0.660	0.565	0.454
Total(T_i)	2.709	2.301	2.641	2.260	1.817

1.3 One way to obtain an estimate of the pooled variance is to construct an ANOVA table including all sums of squares, using the following formulas:

Where: p = number of effluent concentrations including:

$$SST = \sum_{ij} Y_{ij}^2 - G^2/N \qquad \text{Total Sum of Squares}$$

$$SSB = \sum_{i} T_i^2/n_i - G^2/N \qquad \text{Between Sum of Squares}$$

$$SSW = SST - SSB \qquad \text{Within Sum of Squares}$$

G = the grand total of all sample observations; $G = \sum_{i=1}^{P} T_i$

T_i = the total of the replicate measurements for concentration i

N = the total sample size; $N = \sum_i n_i$

n_i = the number of replicates for concentration i

Y_{ij} = the jth observation for concentration i

1.4 For the data in this example:

$n_1 = n_2 = n_3 = n_4 = n_5 = 4$

$N = 20$

$T_1 = Y_{11} + Y_{12} + Y_{13} + Y_{14} = 2.709$
$T_2 = Y_{21} + Y_{22} + Y_{23} + Y_{24} = 2.301$
$T_3 = Y_{31} + Y_{32} + Y_{33} + Y_{34} = 2.641$
$T_4 = Y_{41} + Y_{42} + Y_{43} + Y_{44} = 2.260$
$T_5 = Y_{51} + Y_{52} + Y_{53} + Y_{54} = 1.817$

$G = T_1 + T_2 + T_3 + T_4 + T_5 = 11.728$

$$SST = \sum_{ij} Y_{ij}^2 - G^2/N$$

$$= 7.146 - (11.728)^2/20$$

$$= 0.2687$$

$$SSB = \sum_i T_i^2/n_i - G^2/N$$

$$= \tfrac{1}{4}\,(28.017 - 11.728)^2/20$$

$$= 0.1270$$

$$SSW = SST - SSB$$

$$= 0.2687 - 0.1270$$

$$= 0.1417$$

1.5 Summarize these data in the ANOVA table (Table C.2).

TABLE C.2. ANOVA TABLE FOR DUNNETT'S PROCEDURE

Source	df	Sum of Squares (SS)	Mean Square (MS) (SS/df)
Between	p - 1	SSB	S_B^2 = SSB/(p-1)
Within	N - p	SSW	S_W^2 = SSW/(N-p)
Total	N - 1	SST	

1.6 Summarize data for ANOVA (Table C.3).

TABLE C.3. COMPLETED ANOVA TABLE FOR DUNNETT'S PROCEDURE

Source	df	SS	Mean Square
Between	5 - 1 = 4	0.1270	0.0318
Within	20 - 5 = 15	0.1417	0.0094
Total	19	0.2687	

1.7 To perform the individual comparisons, calculate the t statistic for each concentration and control combination, as follows:

$$t_i = \frac{(\bar{Y}_1 - \bar{Y}_i)}{S_w\sqrt{(1/n_1) + (1/n_i)}}$$

Where: $\bar{Y}_i$ = mean for concentration i

$\bar{Y}_1$ = mean for the control

S_w = square root of the within mean square

n_1 = number of replicates in the control

n_i = number of replicates for concentration i.

1.8 Table C.4 includes the calculated t values for each concentration and control combination.

TABLE C.4. CALCULATED T VALUES

NaPCP Concentration (µg/L)	i	t_i
32	2	1.487
64	3	0.248
128	4	1.633
256	5	3.251

1.9 Since the purpose of the test is only to detect a decrease in growth from the control, a one-sided test is appropriate. The critical value for the one-sided comparison (2.36), with an overall alpha level of 0.05, 15 degrees of freedom and four concentrations excluding the control is read from the table of Dunnett's "T" values (Table C.5; this table assumes an equal number of replicates in all treatment concentrations and the control). The mean weight for concentration i is considered significantly less than the mean weight for the control if t_i is greater than the critical value. Since T_5 is greater than 2.36, the 256 µg/L concentration has significantly lower growth than the control. Hence the NOEC and LOEC for growth are 128 µg/L and 256 µg/L, respectively.

TABLE C.5. DUNNETT'S "T" VALUES (Miller, 1981)

(One-tailed)d^{α}_{k}																		
ν \ k	$\alpha = .05$									$\alpha = 0.1$								
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
5	2.02	2.44	2.58	2.85	2.98	3.08	3.16	3.24	3.30	3.37	3.90	4.21	4.43	4.50	4.73	4.85	4.94	5.03
6	1.94	2.34	2.56	2.71	2.83	2.92	3.00	3.07	3.12	3.14	3.61	4.88	4.07	4.21	4.33	4.43	4.51	4.39
7	1.89	2.27	2.48	2.62	2.73	2.82	2.89	2.95	3.01	3.00	3.42	3.56	3.83	3.96	4.07	4.15	4.23	4.30
8	1.86	2.22	2.42	2.55	2.56	2.74	2.81	2.87	2.92	2.90	3.20	3.51	3.67	3.79	3.18	3.96	4.03	4.09
9	1.83	2.18	2.37	2.50	2.60	2.68	2.75	2.81	2.86	2.82	3.19	3.40	3.55	3.86	3.75	3.82	3.89	3.94
10	1.81	2.15	2.34	2.47	2.56	2.64	2.70	2.76	2.81	2.76	3.11	3.31	3.45	3.56	3.64	3.71	3.78	3.83
11	1.80	2.13	2.31	2.44	2.53	2.60	2.67	2.72	2.77	2.72	3.06	3.25	3.38	3.46	3.56	3.63	3.69	3.74
12	1.78	2.11	2.29	2.41	2.50	2.58	2.64	2.59	2.74	2.68	3.01	3.19	3.32	3.42	3.50	3.56	3.62	3.67
13	1.77	2.09	2.27	2.39	2.48	2.55	2.61	2.68	2.71	2.65	2.97	3.15	3.27	3.37	3.44	3.91	3.56	3.61
14	1.76	2.08	2.25	2.37	2.46	2.53	2.59	2.64	2.69	2.62	2.94	3.11	3.23	3.32	3.40	3.46	3.51	3.56
15	1.75	2.07	2.24	2.36	2.44	2.51	2.57	2.62	2.67	2.60	2.91	3.08	3.20	3.29	3.36	3.42	3.47	3.52
16	1.75	2.06	2.23	2.34	2.43	2.50	2.56	2.61	2.65	2.58	2.38	3.05	3.17	3.28	3.33	3.39	3.44	3.48
17	1.74	2.05	2.22	2.33	2.42	2.49	2.54	2.59	2.64	2.57	2.86	3.03	3.14	3.23	3.30	3.36	3.41	3.45
18	1.73	2.04	2.21	2.32	2.41	2.48	2.53	2.58	2.62	2.55	2.84	3.01	3.12	3.21	3.27	3.33	3.38	3.40
19	1.73	2.03	2.20	2.31	2.40	2.47	2.52	2.57	2.61	2.54	2.83	2.99	3.10	3.18	3.25	3.31	3.36	3.40
20	1.72	2.03	2.19	2.30	2.30	2.46	2.51	2.56	2.60	2.53	2.81	2.97	3.08	3.17	3.23	3.29	3.34	3.40
24	1.71	2.01	3.17	2.28	2.36	2.43	2.48	2.53	2.57	2.40	2.77	2.92	3.03	3.11	3.17	3.22	3.27	3.31
30	1.70	1.99	2.15	2.25	2.33	2.40	2.45	2.50	2.54	2.46	2.72	2.87	2.97	3.05	3.11	3.16	3.21	3.24
40	1.68	1.97	2.13	2.23	2.31	2.37	2.42	2.47	2.51	2.42	2.68	2.32	2.92	2.99	3.06	3.10	3.14	3.18
60	1.67	1.95	2.10	2.21	2.28	2.35	2.39	2.44	2.48	2.39	2.64	2.78	2.87	2.94	3.08	3.04	3.06	3.12
120	1.86	1.93	2.08	2.18	2.26	2.32	2.37	2.41	2.45	2.36	2.60	2.73	2.82	2.90	2.94	2.90	3.03	3.06
α	1.64	1.92	2.06	2.16	2.23	2.29	2.34	2.33	2.42	2.33	2.56	2.68	2.77	2.34	2.90	2.93	2.97	3.00

1.10 To quantify the sensitivity of the test, the minimum significant difference (MSD) may be calculated. The formula is as follows:

$$MSD = d\,S_w\sqrt{(1/n_1) + (1/n)}$$

Where: d = critical value for the Dunnett's Procedure

S_w = the square root of the within mean square

n = the number of replicates at each concentration, assuming an equal number of replicates at all treatment concentrations

n_1 = number of replicates in the control

For example:

$$MSD = 2.36(0.097)[3\sqrt{(1/4)+(1/4)}] = 2.36(0.097)(\sqrt{2/4})$$

= 2.36 (0.097)(0.707)

= 0.162

1.11 For this set of data, the minimum difference between the control mean and a concentration mean that can be detected as statistically significant is 0.087 mg. This represents a decrease in growth of 24% from the control.

1.11.1 If the data have not been transformed, the MSD (and the percent decrease from the control mean that it represents) can be reported as is.

1.11.2 In the case where the data have been transformed, the MSD would be in transformed units. In this case carry out the following conversion to determine the MSD in untransformed units.

1.11.2.1 Subtract the MSD from the transformed control mean. Call this difference D. Next, obtain untransformed values for the control mean and the difference, D.

MSD_u = $control_u$ - D_u

Where: MSD_u = the minimum significant difference for untransformed data

$Control_u$ = the untransformed control mean

D_u = the untransformed difference

1.11.2.2 Calculate the percent reduction from the control that MSD_u represents as:

$$\text{Percent Reduction} = \frac{MSD_u}{Control_u} \times 100$$

1.11.3 An example of a conversion of the MSD to untransformed units, when the arc sine square root transformation was used on the data, follows:

Step 1. Subtract the MSD from the transformed control mean. As an example, assume the data in Table C.1 were transformed by the arc sine square root transformation. Thus:

$$0.677 - 0.162 = 0.515$$

Step 2. Obtain untransformed values for the control mean (0.677) and the difference (0.515) obtained in Step 1 above.

$$[\ \text{Sine}\ (0.677)]^2 = 0.392$$
$$[\ \text{Sine}\ (0.515)]^2 = 0.243$$

Step 3. The untransformed MSD (MSD_u) is determined by subtracting the untransformed values obtained in Step 2.

$$MSD_u = 0.392 - 0.243 = 0.149$$

In this case, the MSD would represent a 38.0% decrease in survival from the control [(0.149/0.392)(100)].

2. COMPUTER CALCULATIONS

2.1 This computer program incorporates two analyses: an analysis of variance (ANOVA), and a multiple comparison of treatment means with the control mean (Dunnett's Procedure). The ANOVA is used to obtain the error value. Dunnett's Procedure indicates which toxicant concentration means (if any) are statistically different from the control mean at the 5% level of significance. The program also provides the minimum difference between the control and treatment means that could be detected as statistically significant, and tests the validity of the homogeneity of variance assumption by Bartlett's Test. The multiple comparison is performed based on procedures described by Dunnett (1955).

2.2 The source code for the Dunnett's program is structured into a series of subroutines, controlled by a driver routine. Each subroutine has a specific function in the Dunnett's Procedure, such as data input, transforming the data, testing for equality of variances, computing p values, and calculating the one-way analysis of variance.

2.3 The program compares up to seven toxicant concentrations against the control, and can accommodate up to 50 replicates per concentration.

2.4 If the number of replicates at each toxicant concentration and control are not equal, a t test with Bonferroni's adjustment is performed instead of Dunnett's Procedure (see Appendix D).

2.5 The program was written in IBM-PC FORTRAN by Computer Sciences Corporation, 26 W. Martin Luther King Drive, Cincinnati, OH 45268. A compiled executable version of the program can be obtained from EMSL-Cincinnati by sending a written request to EMSL at 3411 Church Street, Cincinnati, OH 45244.

2.6 DATA INPUT AND OUTPUT

2.6.1 Reproduction data from a daphnid, *Ceriodaphnia dubia,* survival and reproduction test (Table C.6) are used to illustrate the data input and output for this program.

TABLE C.6. SAMPLE DATA FOR DUNNETT'S PROGRAM *CERIODAPHNIA DUBIA* REPRODUCTION DATA

		Effluent Concentration (%)			
Replicate	Control	1.56	3.12	6.25	12.5
1	27	32	39	27	10
2	30	35	30	34	13
3	29	32	33	36	7
4	31	26	33	34	7
5	16	18	36	31	7
6	15	29	33	27	10
7	18	27	33	33	10
8	17	16	27	31	16
9	14	35	38	33	12
10	27	13	44	31	2

2.6.2 Data Input

2.6.2.1 When the program is entered, the user is asked to select the type of data to be entered:

1. Response proportions, like survival or fertilization proportions.
2. Counts and measurements, like offspring counts, cystocarp counts or weights.

2.6.2.2 After the type of data is chosen, the user has the following options:

1. Create a data file
2. Edit a data file
3. Perform analysis on existing data set
4. Stop

2.6.2.3 When Option 1 (Create a data file) is selected for counts and measurements, the program prompts the user for the following information:

1. Number of concentrations, including control
2. For each concentration:
   - number of observations
   - data for each observation

2.6.2.4 After the data have been entered, the user may save the file on a disk, and the program returns to the menu (see below).

2.6.2.5 Sample data input is shown in Figure C.1.

```
                     EMSL Cincinnati Dunnett Software
                               Version 1.5

      1)    Create a data file
      2)    Edit a data file
      3)    Perform ANOVA on existing data
      4)    Stop

Your choice ? 1


Number of groups, including control ? 5

Number of observations for group  1 ? 10

Enter the data for group  1 one observation at a time.

NO.  1?  27

NO.  2?  30

NO.  3?  29

NO.  4?  31

NO.  5?  16

NO.  6?  15

NO.  7?  18

NO.  8?  17

NO.  9?  14

NO. 10?  27

Number of observations for group  2  ?  10


Do you wish to save the data on disk  ?y

Disk file for output  ?  cerio
```

Figure C.1.   Sample Data Input for Dunnett's Program for Reproduction Data from Table C.6.

2.6.3 Program Output

2.6.3.1 When Option 3 (Perform analysis on existing data set) is selected from the menu, the user is asked to select the transformation desired, and indicate whether they expect the means of the test groups to be less or greater than the mean for the control group (see Figure C.2).

2.6.3.2 Summary statistics (Figure C.3) for the raw and transformed data, if applicable, the ANOVA table, results of Bartlett's Test, the results of the multiple comparison procedure and the minimum detectable difference are included in the program output.

EMSL Cincinnati Dunnett Software
Version 1.5

1) Create a data file
2) Edit a data file
3) Perform analysis on existing data set
4) Stop

Your choice ? 3

File name ? cerio

Available Transformations

1) no transform
2) square root
3) log10

Your choice ? 1

Dunnett's test as implemented in this program is
a one-sided test. You must specify the direction
the test is to be run; that is, do you expect the
means for the test groups to be less than or
greater than the mean for the control group mean.

Direction for Dunnett's test : L=less than, G=greater than ? L

Figure C.2. Example of Choosing Option 3 from the Menu of the Dunnett Program.

Ceriodaphnia Reproduction Data from Table C.6

```
                    Summary Statistics and ANOVA

               Transformation =      None

   Group       n            Mean           s.d.           CV%
________________________________________________________________________

1 = control   10          22.4000         6.9314          30.9
    2         10          26.3000         8.0007          30.4
    3         10          34.6000         4.8351          14.0
    4         10          31.7000         2.9458           9.3
    5*        10           9.4000         3.8930          41.4
________________________________________________________________________

*) the mean for this group is significantly less than the control
   mean at alpha = 0.05 (1-sided) by Dunnett's test

Minimum detectable difference for Dunnett's test = -5.628560
This difference corresponds to -25.13 percent of control

Between concentrations
Sum of squares = 3887.880000 with  4 degrees of freedom.

Error mean square = 31.853333 with 45 degrees of freedom.

Bartlett's test p-value for equality of variances = .029

Do you wish to restart the program ?
```

Figure C.3. Example of Program Output for the Dunnett's Program Using the Reproduction Data from Table C.6.

APPENDIX D

T TEST WITH BONFERRONI'S ADJUSTMENT

1. The t test with Bonferroni's adjustment is used as an alternative to Dunnett's Procedure when the number of replicates is not the same for all concentrations. This test sets an upper bound of alpha on the overall error rate, in contrast to Dunnett's Procedure, for which the overall error rate is fixed at alpha. Thus, Dunnett's Procedure is a more powerful test.

2. The t test with Bonferroni's adjustment is based on the same assumptions of normality and homogeneity of variance as Dunnett's Procedure (see Appendix B for testing these assumptions), and, like Dunnett's Procedure, uses a pooled estimate of the variance, which is equal to the error value calculated in an analysis of variance.

3. An example of the use of the t test with Bonferroni's adjustment is provided below. The data used in the example are the same as in Appendix C, except that the third replicate from the 256 µg/L concentration is presumed to have been lost. Thus, Dunnett's Procedure cannot be used. The weight data are presented in Table D.1.

TABLE D.1. FATHEAD MINNOW, *PIMEPHALES PROMELAS,* LARVAL GROWTH DATA (WEIGHT IN MG) USED FOR THE T-TEST WITH BONFERRONI'S ADJUSTMENT

		NaPCP Concentration (µg/L)			
Replicate	Control	32	64	128	256
A	0.711	0.517	0.602	0.566	0.455
B	0.662	0.501	0.669	0.612	0.502
C	0.646	0.723	0.694	0.410	(LOST)
D	0.690	0.560	0.676	0.672	0.254
Mean($\bar{Y}$)	0.677	0.575	0.660	0.565	0.404
Total(T_i)	2.709	2.301	2.641	2.260	1.211

3.1 One way to obtain an estimate of the pooled variance is to construct an ANOVA table including all sums of squares, using the following formulas:

Where: p = number of effluent concentrations including the control

N = the total sample size; $N = \sum_i n_i$

n_i = the number of replicates for concentration i

$SST = \sum_{ij} Y_{ij}^2 - G^2/N$ Total Sum of Squares

$$SSB = \sum_i T_i^2/n_i - G^2/N$$ Between Sum of Squares

$$SSW = SST - SSB$$ Within Sum of Squares

Where: G = The grand total of all sample observations; $G = \sum_{i=1}^{P} T_i$

T_i = The total of the replicate measurements for concentration i

Y_{ij} = The jth observation for concentration i

3.2 For the data in this example:

$n_1 = n_2 = n_3 = n_4 = 4$

$T_1 = Y_{11} + Y_{12} + Y_{13} + Y_{14} = 2.709$
$T_2 = Y_{21} + Y_{22} + Y_{23} + Y_{24} = 2.301$
$T_3 = Y_{31} + Y_{32} + Y_{33} + Y_{34} = 2.641$
$T_4 = Y_{41} + Y_{42} + Y_{43} + Y_{44} = 2.260$
$T_5 = Y_{51} + Y_{52} + Y_{53} + Y_{54} = 1.211$
$G = T_1 + T_2 + T_3 + T_4 + T_5 = 11.122$

$$SSB = \sum_i T_i^2/n_i - G^2/N$$

$= 6.668 - (11.122)^2/19$

$= 0.158$

$$SST = \sum_{ij} Y_{ij}^2 - G^2/N$$

$= 6.779 - (11.122)^2/19$

$= 0.269$

$$SSW = SST - SSB$$

$= \quad 0.269 - 0.158$

$= \quad 0.111$

3.3 Summarize these data in the ANOVA table (Table D.2):

TABLE D.2. ANOVA TABLE FOR BONFERRONI'S ADJUSTMENT

Source	df	Sum of Squares (SS)	Mean Square (MS) (SS/df)
Between	p - 1	SSB	$S_B^2 = SSB/(p-1)$
Within	N - p	SSW	$S_W^2 = SSW/(N-p)$
Total	N - 1	SST	

3.4 Summarize these data in the ANOVA table (Table D.3):

TABLE D.3. COMPLETED ANOVA TABLE FOR THE T-TEST WITH BONFERRONI'S ADJUSTMENT

Source	df	SS	Mean Square
Between	5 - 1 = 4	0.158	0.0395
Within	19 - 5 = 14	0.111	0.0029
Total	18	0.269	

3.5 To perform the individual comparisons, calculate the t statistic for each concentration and control combination, as follows:

$$t_i = \frac{(\bar{Y}_1 - \bar{Y}_i)}{S_w\sqrt{(1/n_1) + (1/n_i)}}$$

Where: $\bar{Y}_i$ = mean for each concentration

$\bar{Y}_1$ = mean for the control

S_w = square root of the within mean square

n_1 = number of replicates in the control.

n_i = number of replicates for concentration i.

3.6 Table D.4 includes the calculated t values for each concentration and control combination.

TABLE D.4. CALCULATED T VALUES

NaPCP Concentration (µg/L)	i	t_i
32	2	1.623
64	3	0.220
128	4	1.782
256	5	4.022

3.7 Since the purpose of the test is only to detect a decrease in growth from the control, a one-sided test is appropriate. The critical value for the one-sided comparison (2.510), with an overall alpha level of 0.05, fourteen degrees of freedom and four concentrations excluding the control, was obtained from Table D.5. The mean weight for concentration "i" is considered significantly less than the mean weight for the control if t_i is greater than the critical value. Since t_5 is greater than 2.510, the 256 µg/L concentration has significantly lower growth than the control. Hence the NOEC and LOEC for growth are 128 µg/L and 256 µg/L, respectively.

TABLE D.5. CRITICAL VALUES FOR "T" FOR THE T TEST WITH BONFERRONI'S ADJUSTMENT
P = 0.05 CRITICAL LEVEL, ONE TAILED

df	K = 1	K = 2	K = 3	K = 4	K = 5	K = 6	K = 7	K = 8	K = 9	K = 10
1	6.314	12.707	19.002	25.452	31.821	38.189	44.556	50.924	57.290	63.657
2	2.920	4.303	5.340	6.206	6.965	7.649	8.277	8.861	9.408	9.925
3	2.354	3.183	3.741	4.177	4.541	4.857	5.138	5.392	5.626	5.841
4	2.132	2.777	3.187	3.496	3.747	3.961	4.148	4.315	4.466	4.605
5	2.016	2.571	2.912	3.164	3.365	3.535	3.681	3.811	3.927	4.033
6	1.944	2.447	2.750	2.969	3.143	3.288	3.412	3.522	3.619	3.708
7	1.895	2.365	2.642	2.842	2.998	3.128	3.239	3.336	3.422	3.500
8	1.860	2.307	2.567	2.752	2.897	3.016	3.118	3.206	3.285	3.356
9	1.834	2.263	2.510	2.686	2.822	2.934	3.029	3.111	3.185	3.250
10	1.813	2.229	2.406	2.634	2.764	2.871	2.961	3.039	3.108	3.170
11	1.796	2.301	2.432	2.594	2.719	2.821	2.907	2.981	3.047	3.106
12	1.783	2.179	2.404	2.561	2.681	2.730	2.863	2.935	2.998	3.055
13	1.771	2.161	2.380	2.533	2.651	2.746	2.827	2.897	2.950	3.013
14	1.762	2.145	2.360	2.510	2.625	2.718	2.797	2.864	2.924	2.977
15	1.754	2.132	2.343	2.490	2.603	2.694	2.771	2.837	2.895	2.947
16	1.746	2.120	2.329	2.473	2.584	2.674	2.749	2.814	2.871	2.921
17	1.740	2.110	2.316	2.459	2.567	2.655	2.729	2.793	2.849	2.899
18	1.735	2.101	2.305	2.446	2.553	2.640	2.712	2.775	2.830	2.879
19	1.730	2.094	2.295	2.434	2.540	2.626	2.697	2.759	2.813	2.861
20	1.725	2.086	2.206	2.424	2.528	2.613	2.684	2.745	2.798	2.846
21	1.721	2.080	2.278	2.414	2.518	2.602	2.672	2.732	2.785	2.832
22	1.718	2.074	2.271	2.406	2.509	2.592	2.661	2.721	2.773	2.819
23	1.714	2.069	2.264	2.398	2.500	2.583	2.651	2.710	2.762	2.808
24	1.711	2.064	2.258	2.391	2.493	2.574	2.642	2.701	2.752	2.797
25	1.709	2.060	2.253	2.385	2.486	2.566	2.634	2.692	2.743	2.788
26	1.706	2.056	2.248	2.379	2.479	2.559	2.627	2.684	2.734	2.779
27	1.704	2.052	2.243	2.374	2.473	2.553	2.620	2.677	2.727	2.771
28	1.702	2.049	2.239	2.369	2.468	2.547	2.613	2.670	2.720	2.764

TABLE D.5. CRITICAL VALUES FOR "T" FOR THE T TEST WITH BONFERRONI'S ADJUSTMENT
P = 0.05 CRITICAL LEVEL, ONE TAILED (CONTINUED)

df	K = 1	K = 2	K = 3	K = 4	K = 5	K = 6	K = 7	K = 8	K = 9	K = 10
29	1.700	2.046	2.235	2.364	2.463	2.541	2.607	2.664	2.713	2.757
30	1.698	2.043	2.231	2.360	2.458	2.536	2.602	2.658	2.707	2.750
31	1.696	2.040	2.228	2.356	2.453	2.531	2.597	2.652	2.701	2.745
32	1.694	2.037	2.224	2.352	2.449	2.527	2.592	2.647	2.696	2.739
33	1.693	2.035	2.221	2.349	2.445	2.523	2.587	2.643	2.691	2.734
34	1.691	2.033	2.219	2.346	2.442	2.519	2.583	2.638	2.686	2.729
35	1.690	2.031	2.216	2.342	2.438	2.515	2.579	2.634	2.682	2.724
36	1.689	2.029	2.213	2.340	2.435	2.512	2.575	2.630	2.678	2.720
37	1.688	2.027	2.211	2.337	2.432	2.508	2.572	2.626	2.674	2.716
38	1.686	2.025	2.209	2.334	2.429	2.505	2.568	2.623	2.670	2.712
39	1.685	2.023	2.207	2.332	2.426	2.502	2.565	2.619	2.667	2.708
40	1.684	2.022	2.205	2.329	2.424	2.499	2.562	2.616	2.663	2.705
50	1.676	2.009	2.189	2.311	2.404	2.478	2.539	2.592	2.638	2.678
60	1.671	2.001	2.179	2.300	2.391	2.463	2.324	2.576	2.621	2.661
70	1.667	1.995	2.171	2.291	2.381	2.453	2.513	2.564	2.609	2.648
80	1.665	1.991	2.166	2.285	2.374	2.446	2.505	2.556	2.600	2.639
90	1.662	1.987	2.162	2.280	2.369	2.440	2.499	2.549	2.593	2.632
100	1.661	1.984	2.158	2.276	2.365	2.435	2.494	2.544	2.588	2.626
110	1.659	1.982	2.156	2.273	2.361	2.432	2.490	2.540	2.583	2.622
120	1.658	1.980	2.153	2.270	2.358	2.429	2.487	2.536	2.580	2.618
Infinite	1.645	1.960	2.129	2.242	2.327	2.394	2.450	2.498	2.540	2.576

d.f. = Degrees of freedom for MSE (Mean Square Error) from ANOVA.
K = Number of concentrations to be compared to the control.

APPENDIX E

STEEL'S MANY-ONE RANK TEST

1. Steel's Many-one Rank Test is a nonparametric test for comparing treatments with a control. This test is an alternative to Dunnett's Procedure, and may be applied to data when the normality assumption has not been met. Steel's Test requires equal variances across the treatments and the control, but it is thought to be fairly insensitive to deviations from this condition (Steel, 1959). The tables for Steel's Test require an equal number of replicates at each concentration. If this is not the case, use Wilcoxon's Rank Sum Test, with Bonferroni's adjustment (see Appendix F).

2. For an analysis using Steel's Test, for each control and concentration combination, combine the data and arrange the observations in order of size from smallest to largest. Assign the ranks to the ordered observations (1 to the smallest, 2 to the next smallest, etc.). If ties occur in the ranking, assign the average rank to the observation. (Extensive ties would invalidate this procedure). The sum of the ranks within each concentration and within the control is then calculated. To determine if the response in a concentration is significantly different from the response in the control, the minimum rank sum for each concentration and control combination is compared to the significant values of rank sums given later in this section. In this table, k equals the number of treatments excluding the control and n equals the number of replicates for each concentration and the control.

3. An example of the use of this test is provided below. The test employs reproduction data from a *Ceriodaphnia dubia* 7-day, chronic test. The data are listed in Table E.1. Significant mortality was detected via Fisher's Exact Test in the 50% effluent concentration. The data for this concentration is not included in the reproduction analysis.

TABLE E.1. EXAMPLE OF STEEL'S MANY-ONE RANK TEST: DATA FOR THE DAPHNID, *CERIODAPHNIA DUBIA*, 7-DAY CHRONIC TEST

Effluent Concentration	Replicate										No. Live Adults
	1	2	3	4	5	6	7	8	9	10	
Control	20	26	26	23	24	27	26	23	27	24	10
3%	13	15	14	13	23	26	0	25	26	27	9
6%	18	22	13	13	23	22	20	22	23	22	10
12%	14	22	20	23	20	23	25	24	25	21	10
25%	9	0	9	7	6	10	12	14	9	13	8
50%	0	0	0	0	0	0	0	0	0	0	0

4. For each control and concentration combination, combine the data and arrange the observations in order of size from smallest to largest. Assign ranks (1, 2, 3,..., 16) to the ordered observations (1 to the smallest, 2 to the next smallest, etc.). If ties occur in the ranking, assign the average rank to each tied observation.

5. An example of assigning ranks to the combined data for the control and 3% effluent concentration is given in Table E.2. This ranking procedure is repeated for each control and concentration combination. The complete set of rankings is listed in Table E.3. The ranks are then summed for each effluent concentration, as shown in Table E.4.

TABLE E.2. EXAMPLE OF STEEL'S MANY-ONE RANK TEST: ASSIGNING RANKS TO THE CONTROL AND 3% EFFLUENT CONCENTRATION

Rank	Number of Young Produced	Control or % Effluent
1	0	3
2.5	13	3
2.5	13	3
4	14	3
5	15	3
6	20	Control
8	23	Control
8	23	Control
8	23	3
10.5	24	Control
10.5	24	Control
12	25	3
15	26	Control
15	26	Control
15	26	Control
15	26	3
15	26	3
19	27	Control
19	27	Control
19	27	3

TABLE E.3. TABLE OF RANKS

Replicate (Organism)	Control[1]	Effluent Concentration (%) 3	6	12	25
1	20 (6,4.5,3,11)	13 (2.5)	18 (3)	14 (1)	9 (5)
2	26 (15,17,17,17)	15 (5)	22 (7.5)	22 (6)	0 (1)
3	26 (15,17,17,17)	14 (4)	13 (1.5)	20 (3)	9 (5)
4	23 (8,11.5,8.5,12.5)	13 (2.5)	13 (1.5)	23 (8.5)	7 (3)
5	24 (10.5,14.5,12,14.5)	23 (8)	23 (11.5)	20 (3)	6 (2)
6	27 (19,19.5,19.5,19.5)	26 (15)	22 (7.5)	23 (8.5)	10 (7)
7	26 (15,17,17,17)	0 (1)	20 (4.5)	25 (14.5)	12 (8)
8	23 (8,11.5,8.5,12.5)	25 (12)	22 (7.5)	24 (12)	14 (10)
9	27 (19,19.5,19.5,19.5)	26 (15)	23 (11.5)	25 (14.5)	9 (5)
10	24 (10.5,14.5,12,14.5)	27 (19)	22 (7.5)	21 (5)	13 (9)

[1] Control ranks are given in the order of the concentration with which they were ranked.

TABLE E.4. RANK SUMS

Effluent Concentration (%)	Rank Sum
3	84
6	64
12	76
25	55

6. For this set of data, determine if the reproduction in any of the effluent concentrations is significantly lower than the reproduction by the control organisms. If this occurs, the rank sum at that concentration would be significantly lower than the rank sum of the control. Thus, compare the rank sums for the reproduction of each of the various effluent concentrations with some "minimum" or critical rank sum, at or below which the reproduction would be considered to be significantly lower than the control. At a probability level of 0.05, the critical rank in a test with four concentrations and ten replicates is 76 (see Table E.5 , for R=4).

7. Comparing the rank sums in Table E.4 to the appropriate critical rank, the 6%, 12% and 25% effluent concentrations are found to be significantly different from the control. Thus the NOEC and LOEC for reproduction are 3% and 6%, respectively.

TABLE E.5. SIGNIFICANT VALUES OF RANK SUMS: JOINT CONFIDENCE COEFFICIENTS OF 0.95 (UPPER) and 0.99 (LOWER) FOR ONE-SIDED ALTERNATIVES (Steel, 1959)

	k = number of treatments (excluding control)							
n	2	3	4	5	6	7	8	9
4	11	10	10	10	10	--	--	--
	--	--	--	--	--	--	--	--
5	18	17	17	16	16	16	16	15
	15	--	--	--	--	--	--	--
6	27	26	25	25	24	24	24	23
	23	22	21	21	--	--	--	--
7	37	36	35	35	34	34	33	33
	32	31	30	30	29	29	29	29
8	49	48	47	46	46	45	45	44
	43	42	41	40	40	40	39	39
9	63	62	61	60	59	59	58	58
	56	55	54	53	52	52	51	51
10	79	77	76	75	74	74	73	72
	71	69	68	67	66	66	65	65
11	97	95	93	92	91	90	90	89
	87	85	84	83	82	81	81	80
12	116	114	112	111	110	109	108	108
	105	103	102	100	99	99	98	98
13	138	135	133	132	130	129	129	128
	125	123	121	120	119	118	117	117
14	161	158	155	154	153	152	151	150
	147	144	142	141	140	139	138	137
15	186	182	180	178	177	176	175	174
	170	167	165	164	162	161	160	160
16	213	209	206	204	203	201	200	199
	196	192	190	188	187	186	185	184
17	241	237	234	232	231	229	228	227
	223	219	217	215	213	212	211	210
18	272	267	264	262	260	259	257	256
	252	248	245	243	241	240	239	238
19	304	299	296	294	292	290	288	287
	282	278	275	273	272	270	268	267
20	339	333	330	327	325	323	322	320
	315	310	307	305	303	301	300	299

APPENDIX F

WILCOXON RANK SUM TEST

1. Wilcoxon's Rank Sum Test is a nonparametric test, to be used as an alternative to Steel's Many-one Rank Test when the number of replicates are not the same at each concentration. A Bonferroni's adjustment of the pairwise error rate for comparison of each concentration versus the control is used to set an upper bound of alpha on the overall error rate, in contrast to Steel's Many-one Rank Test, for which the overall error rate is fixed at alpha. Thus, Steel's Test is a more powerful test.

2. An example of the use of the Wilcoxon Rank Sum Test is provided in Table F.1. The data used in the example are the same as in Appendix E, except that two males are presumed to have occurred, one in the control and one in the 12% effluent concentration. Thus, there is unequal replication for the reproduction analysis.

3. For each concentration and control combination, combine the data and arrange the values in order of size, from smallest to largest. Assign ranks to the ordered observations (a rank of 1 to the smallest, 2 to the next smallest, etc.). If ties in rank occur, assign the average rank to each tied observation.

TABLE F.1. EXAMPLE OF WILCOXON'S RANK SUM TEST: DATA FOR THE DAPHNID, *CERIODAPHNIA DUBIA*, 7-DAY CHRONIC TEST

Effluent	Replicate										No. Live
Concentration	1	2	3	4	5	6	7	8	9	10	Adults
Cont	M	26	26	23	24	27	26	23	27	24	10
3%	13	15	14	13	23	26	0	25	26	27	9
6%	18	22	13	13	23	22	20	22	23	22	10
12%	14	22	20	23	M	23	25	24	25	21	10
25%	9	0	9	7	6	10	12	14	9	13	8
50%	0	0	0	0	0	0	0	0	0	0	0

4. An example of assigning ranks to the combined data for the control and 3% effluent concentration is given in Table F.2. This ranking procedure is repeated for each of the three remaining control versus test concentration combinations. The complete set of ranks is listed in Table F.3. The ranks are then summed for each effluent concentration, as shown in Table F.4.

5. For this set of data, determine if the reproduction in any of the effluent concentrations is significantly lower than the reproduction by the control organisms. If this occurs, the rank sum at that concentration would be significantly lower than the rank sum for the control. Thus, compare the rank sums for the reproduction of each of the various effluent concentrations with some "minimum" or critical rank sum, at or below which the reproduction would be considered to be significantly lower than the control. At a probability level of 0.05, the critical rank in a test with four concentrations and nine replicates in the control is 72 for those concentrations with ten replicates, and 60 for those concentrations with nine replicates (see Table F.5, for K = 4).

6. Comparing the rank sums in Table F.4 to the appropriate critical rank, the 6%, 12% and 25% effluent concentrations are found to be significantly different from the control. Thus, the NOEC and LOEC for reproduction are 3% and 6%, respectively.

TABLE F.2. EXAMPLE OF WILCOXON'S RANK SUM TEST: ASSIGNING RANKS TO THE CONTROL AND EFFLUENT CONCENTRATIONS

Rank	Number of Young Produced	Control or % Effluent
1	0	3
2.5	13	3
2.5	13	3
4	14	3
5	15	3
7	23	Control
7	23	Control
7	23	3
9.5	24	Control
9.5	24	Control
11	25	3
14	26	Control
14	26	Control
14	26	Control
14	26	3
14	26	3
18	27	Control
18	27	Control
18	27	3

TABLE F.3. TABLE OF RANKS

Replicate (Organism)	Control[1]	Effluent Concentration (%) 3	6	12	25
1	M	13 (2.5)	18 (3)	14 (1)	9 (5)
2	26 (14,16,15,16)	15 (5)	22 (6.5)	22 (4)	0 (1)
3	26 (14,16,15,16)	14 (4)	13 (1.5)	20 (2)	9 (5)
4	23 (7,10.5,6.5,11.5)	13 (2.5)	13 (1.5)	23 (6.5)	7 (3)
5	24 (9.5,13.5,10,13.5)	23 (7)	23 (10.5)	M	6 (2)
6	27 (18,18.5,17.5,18.5)	26 (14)	22 (6.5)	23 (6.5)	10 (7)
7	26 (14,16,15,16)	0 (1)	20 (4)	25 (12.5)	12 (8)
8	23 (7,10.5,6.5,11.5)	25 (11)	22 (6.5)	24 (10)	14 (10)
9	27 (18,18.5,17.5,18.5)	26 (14)	23 (10.5)	25 (12.5)	9 (5)
10	24 (9.5,13.5,10,13.5)	27 (18)	22 (6.5)	21 (3)	13 (9)

[1] Control ranks are given in the order of the concentration with which they were ranked.

TABLE F.4. RANK SUMS

Effluent Concentration	Rank Sum	No. of Replicates	Critical Rank Sum
3	79	10	72
6	57	10	72
12	58	9	60
25	55	10	72

TABLE F.5. CRITICAL VALUES FOR WILCOXON'S RANK SUM TEST WITH BONFERRONI'S ADJUSTMENT OF ERROR RATE FOR COMPARISON OF "K" TREATMENTS VERSUS A CONTROL FIVE PERCENT CRITICAL LEVEL (ONE-SIDED ALTERNATIVE: TREATMENT CONTROL)

K	No. Replicates in Control	No. of Replicates Per Effluent Concentration							
		3	4	5	6	7	8	9	10
1	3	6	10	16	23	30	39	49	59
	4	6	11	17	24	32	41	51	62
	5	7	12	19	26	34	44	54	66
	6	8	13	20	28	36	46	57	69
	7	8	14	21	29	39	49	60	72
	8	9	15	23	31	41	51	63	72
	9	10	16	24	33	43	54	66	79
	10	10	17	26	35	45	56	69	82
2	3	--	--	15	22	29	38	47	58
	4	--	10	16	23	31	40	49	60
	5	6	11	17	24	33	42	52	63
	6	7	12	18	26	34	44	55	66
	7	7	13	20	27	36	46	57	69
	8	8	14	21	29	38	49	60	72
	9	8	14	22	31	40	51	62	75
	10	9	15	23	32	42	53	65	78
3	3	--	--	--	21	29	37	46	57
	4	--	10	16	22	30	39	48	59
	5	--	11	17	24	32	41	51	62
	6	6	11	18	25	33	43	53	65
	7	7	12	19	26	35	45	56	68
	8	7	13	20	28	37	47	58	70
	9	7	13	21	29	39	49	61	73
	10	8	14	22	31	41	51	63	76

TABLE F.5. CRITICAL VALUES FOR WILCOXON'S RANK SUM TEST WITH BONFERRONI'S ADJUSTMENT OF ERROR RATE FOR COMPARISON OF "K" TREATMENTS VERSUS A CONTROL FIVE PERCENT CRITICAL LEVEL (ONE-SIDED ALTERNATIVE: TREATMENT CONTROL) (CONTINUED)

K	No. Replicates in Control	No. of Replicates Per Effluent Concentration							
		3	4	5	6	7	8	9	10
4	3	--	--	--	21	28	37	46	56
	4	--	--	15	22	30	38	48	59
	5	--	10	16	23	31	40	50	61
	6	6	11	17	24	33	42	52	64
	7	6	12	18	26	34	44	55	67
	8	7	12	19	27	36	46	57	69
	9	7	13	20	28	38	48	60	72
	10	7	14	21	30	40	50	62	75
5	3	--	--	--	--	28	36	46	56
	4	--	--	15	22	29	38	48	58
	5	--	10	16	23	31	40	50	61
	6	--	11	17	24	32	42	52	63
	7	6	11	18	25	34	43	54	66
	8	6	12	19	27	35	45	56	68
	9	7	13	20	28	37	47	59	71
	10	7	13	21	29	39	49	61	74
6	3	--	--	--	--	28	36	45	56
	4	--	--	15	21	29	38	47	58
	5	--	10	16	22	30	39	49	60
	6	--	11	16	24	32	41	51	63
	7	6	11	17	25	33	43	54	65
	8	6	12	18	26	35	45	56	68
	9	6	12	19	27	37	47	58	70
	10	7	13	20	29	38	49	60	73
7	3	--	--	--	--	--	36	45	56
	4	--	--	--	21	29	37	47	58
	5	--	--	15	22	30	39	49	60
	6	--	10	16	23	32	41	51	62
	7	--	11	17	25	33	43	53	65
	8	6	11	18	26	35	44	55	67
	9	6	12	19	27	36	46	58	70
	10	7	13	20	28	38	48	60	72

TABLE F.5. CRITICAL VALUES FOR WILCOXON'S RANK SUM TEST WITH BONFERRONI'S ADJUSTMENT OF ERROR RATE FOR COMPARISON OF "K" TREATMENTS VERSUS A CONTROL FIVE PERCENT CRITICAL LEVEL (ONE-SIDED ALTERNATIVE: TREATMENT CONTROL) (CONTINUED)

K	No. Replicates in Control	No. of Replicate Per Effluent Concentration							
		3	4	5	6	7	8	9	10
8	3	--	--	--	--	--	36	45	55
	4	--	--	--	21	29	37	47	57
	5	--	--	15	22	30	39	49	59
	6	--	10	16	23	31	40	51	62
	7	--	11	17	24	33	42	53	64
	8	6	11	18	25	34	44	55	67
	9	6	12	19	27	36	46	57	69
	10	6	12	19	28	37	48	59	72
9	3	--	--	--	--	--	--	45	55
	4	--	--	--	21	28	37	46	57
	5	--	--	15	22	30	39	48	59
	6	--	10	16	23	31	40	50	62
	7	--	10	17	24	33	42	52	64
	8	--	11	18	25	34	44	55	66
	9	6	11	18	26	35	46	57	69
	10	6	12	19	28	37	47	59	71
10	3	--	--	--	--	--	--	45	55
	4	--	--	--	21	28	37	46	57
	5	--	--	15	22	29	38	48	59
	6	--	10	16	23	31	40	50	61
	7	--	10	16	24	32	42	52	64
	8	--	11	17	25	34	43	54	66
	9	6	11	18	26	35	45	56	68
	10	6	12	19	27	37	47	58	71